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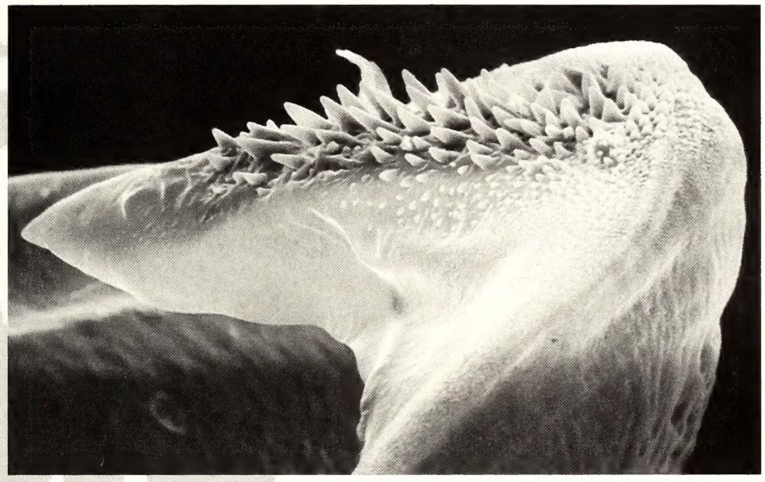

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Pond molluscs of Indiana Dunes National Lakeshore: Then and now*

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Abstract: This investigation documents the molluscan community currently inhabiting ponds and temporary habitats in Indiana Dunes National Lakeshore, Indiana, USA, and compares that fauna to communities described by Shelford (1913) in a successional series of interdunal ponds paralleling the Lake Michigan shoreline nearby. Some molluscan species have remained over the last century, including *Musculium securis, Musculium partumeium, Amnicola limosa, Stagnicola reflexa, Physa gyrina, Planorbella trivolvis, Promenetus exacuous*, and *Planorbula armigera*. In contrast, *Cincinnatia cincinnatiensis, Gyraulus deflectus, Helisoma anceps, Planorbella campanulatum* and three species of unionid mussels were absent from the new survey. *Musculium lacustre, Pisidium casertanum, Valvata tricarinata, Gyraulus parvus, Ferrissia parallela*, and *Laevapex fuscus* were newly discovered. In intermittent habitats, *Stagnicola elodes* replaced the permanent pond dweller *S. reflexa*, and *Aplexa elongata* replaced *Physa gyrina*. Despite the apparently high level of faunal turnover and a general reduction in species richness since 1913, the persistence of a characteristic successional pattern involving *P. armigera, P. exacuous*, and *P. trivolvis* suggests that communities of freshwater gastropods may show some minimal structure.

Key words: Molluscs, Indiana, dune ponds, Shelford

Although the concept of community succession has been fundamental to the study of plant ecology since the inception of the discipline, the application of successional principles to the study of animal ecology has been infrequent. Workers have typically judged successional stage indirectly in freshwater molluscan communities using species richness, and examined the addition or subtraction of species in communities of varying richness for signs of pattern or structure (Dillon 2000). Boycott's (1936) "hierarchy of gentility" in sphaeriid clams is a ranking of 12 species of *Pisidium* Pfeiffer, 1821 by their order of occurrence in succession, as is Jokinen's (1987) analysis of "incidence categories" in the freshwater gastropod fauna of Connecticut. The striking variation in colonization ability displayed by Connecticut freshwater gastropods was interpreted by Dillon (2000) as evidence for his Undifferentiated Stress Tolerance Reproductive Model (USR) of life history variation in which competition is not the main driving factor of the animal's life history pattern.

Among the most influential studies of succession in animal communities has been that of Shelford (1913) on the aquatic fauna of interdunal ponds on the southern shore of Lake Michigan. These ponds, oriented in 95 rows parallel to the modern shoreline, were left behind as the lake receded since the last glaciation, and vary in age from some hundreds to several thousands of years from north to south away from the lake. Shelford described a phenomenon that is in some sense the reverse of succession as it typically occurs in plant communities: The aquatic fauna of the largest, youngest, and mid-aged ponds rivaled Lake Michigan in its diversity, then decreased in diversity as the ponds aged and filled. Molluscs were a prominent component of the biota, including three unionid mussel species, two species of sphaeriid clams, and thirteen species of freshwater gastropods in total.

Shelford's ponds have largely been destroyed by industrial development. Fortunately, a similar series of interdunal ponds has been preserved as a part of the Indiana Dunes National Lakeshore 10 km to the east of Shelford's site (Fig. 1). This affords an opportunity to replicate Shelford's study in a similar environment, independent of the original in both time and space. If succession is a function of chance colonization, weather, environmental perturbation, and similarly nondeterministic factors, one might expect little correspondence between Shelford's observations and the present situation at Indiana Dunes. But to the extent that succession is a function of the life history of the freshwater molluscan fauna, the community interactions between them, and similarly deterministic factors, the observations of Shelford should be confirmed.

The purpose of this study was to document the molluscan communities of Indiana Dunes National Lakeshore, Indiana, USA, and to compare the results obtained with those gathered 90 years ago by V. E. Shelford in a similar series of

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* From the symposium "The Biology and Conservation of Freshwater Gastropods" presented at the annual meeting of the American Malacological Society, held 3-7 August 2002 in Charleston, South Carolina, USA.

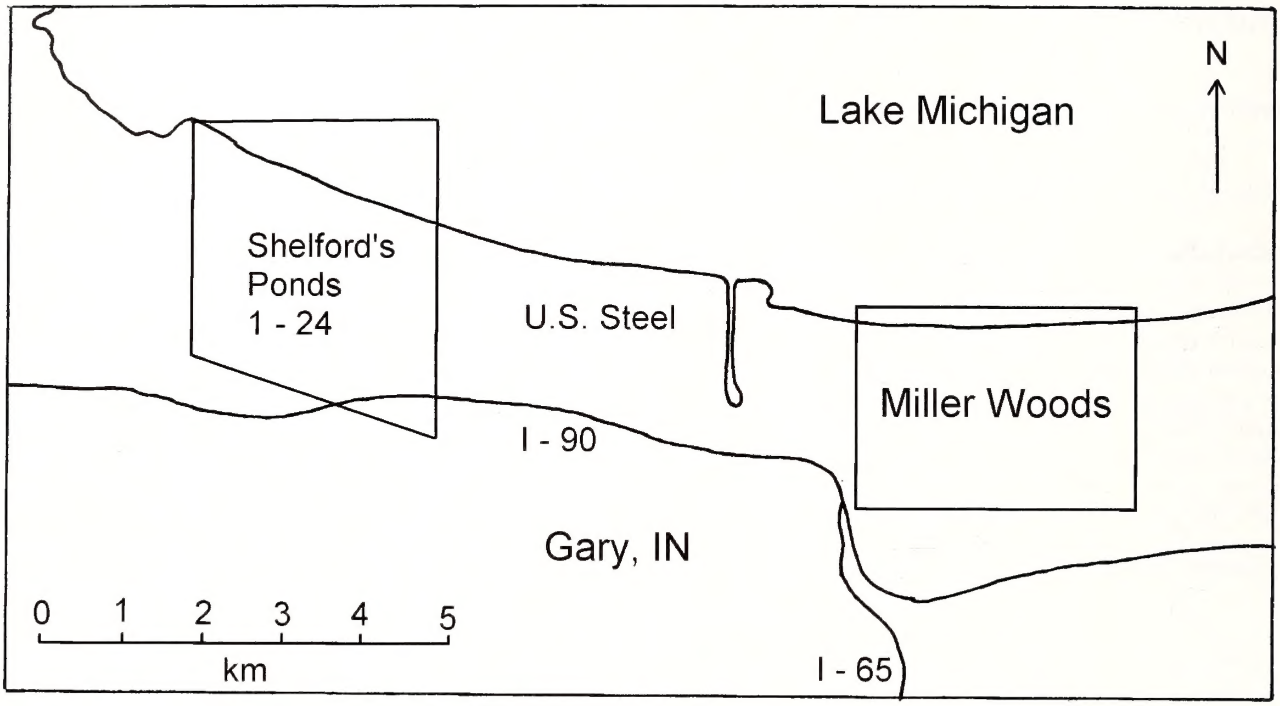

Figure 1. The locations of the first 24 of Shelford's (1913) interdunal ponds (see Fig. 2) and of the Miller Woods section of Indiana Dunes National Lakeshore (see Fig. 3).

dune ponds on the western edge of the city of Gary, Indiana. The National Lakeshore survey included inter- and intradune ponds in Miller Woods and temporary habitats at Cowles Bog, the Huron Rookery, and one railroad ditch outside the park. Although the correspondence between the present environment at Indiana Dunes and the environment sampled by Shelford is a close one, the number of distinct pond rows in much lower at Indiana Dunes, and the number of ponds per row much greater. Thus a description of the methods used in the present study and the results obtained will be preceded by a general overview of the two habitats sampled.

SHELFORD'S PONDS

Shelford's (1913) study area consisted of a series of ponds parallel to the Lake Michigan shoreline, separated by sand ridges averaging 30 m in width (Fig. 2). Ponds varied in depth from a few centimeters to two meters, were somewhat less than 30 m wide, and measured several kilometers in length. Shelford did not sample every pond of the 95 rows paralleling the south shore of Lake Michigan. He omitted some ponds and sampled (north to south) rows 1, 5, 7, 14, 30, 52, 93, and 95. Older pond rows farther south had filled in with terrestrial vegetation. With a gradual fall in lake level, the Grand Calumet River cut through the ridges to flow across the ponds at an acute angle.

Shelford Pond 1 (SP 1) was the youngest and nearest pond to Lake Michigan. The substrate was primarily bare sand. Patches of the macroalga *Chara* sp. (stonewort), were present and served as substrate for the dominant midge larvae, *Chironomus* spp. A number of Lake Michigan fish were present. Molluscs included three species of unionid clams, *Lampsilis siliquoidea* (Barnes, 1823), *Anodonta grandis* (Say, 1829), and *Alasmidonta marginata* (Say, 1819); the gill-breathing hydrobiid snails, *Amnicola limosa* (Say, 1817) and *Cincinnatia cincinnatiensis*(Anthony, 1840); sparse numbers of juvenile individuals of *Physa* sp. and *Fossaria* spp.; and four species of planorbids, *Gyraulus deflectus* (Say, 1824), *Gyraulus parvus* (Say, 1817), *Helisoma anceps* (Menke, 1830), and *Planorbella campanulatum* (Say, 1821). Sphaeriid clams were absent.

Shelford Pond 5 (SP 5) contained a mixture of three communities: (1) bare sand substrate, (2) patches of *Chara* sp., and (3) submerged and floating macrophytes including *Myriophyllum* sp., *Potamogeton* sp., and water lilies. The sphaeriid *Musculium partumeium* (Say, 1822) (= *Musculium truncatum* [Linsley, 1848]), a vegetation dweller also appeared. *Lampsilis siliquoidea* was absent. The remainder of the molluscan community was similar to that of SP 1.

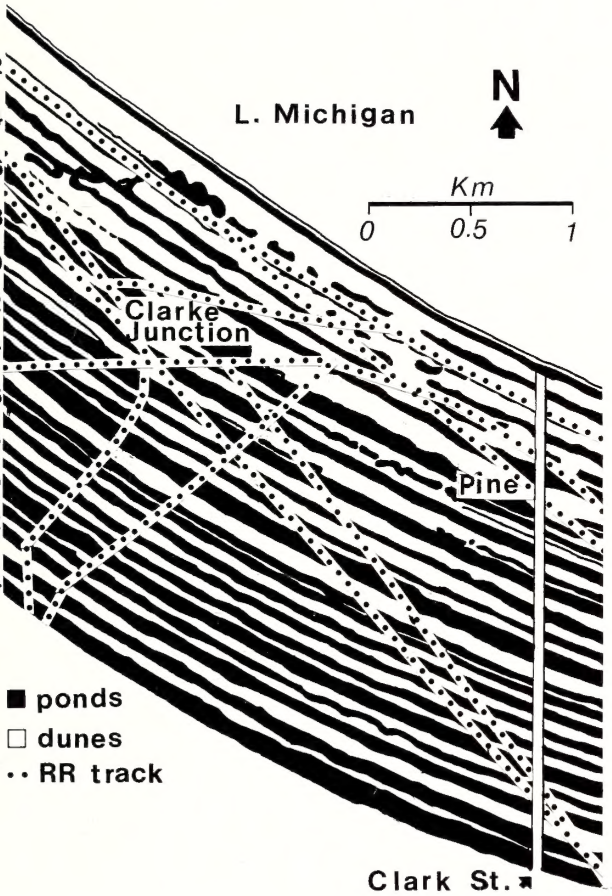

Figure 2. The first 24 parallel, interdunal ponds of Shelford's (1913) study. Pine and Clarke Junction were rail stations used as debarkation points after Shelford 1913: fig. 84).

Shelford Pond 7 (SP 7) had no remaining sand substrate but contained *Chara* sp. and the macrophyte associations. The sphaeriids *Musculium securis* (Prime, 1851) and *Musculium partumeium*, two unionid species, and *Amnicola limosa* were present. *Helisoma anceps* was absent. The two aforementioned unionids were joined by *Alasmidonta* (= *Anodonta*) *marginata*.

Shelford Pond 14 (SP 14) had a mature surface-reaching macrophyte association, within which *Planorbella campanulatum* was the dominant mollusc. Hydrobiids were still present (indicating adequate oxygen), but the small amphibious lymnaeids *Fossaria modicella*(Say, 1825) and *Fossaria obrussa* (Say, 1825) were absent. All unionids were absent. The planorbids, *Planorbella trivolvis* (Say, 1816) and *Planorbula armigera* (Say, 1821) had joined the community.

Shelford Pond 30 (SP 30) also contained a mature surface-reaching macrophyte association. Both sphaeriids were present. *Gyraulus parvus* and *Planorbella campanulatum* were absent, as well as the hydrobiids. The large stagnicoline lymnaeid *Stagnicola reflexa* (Say, 1821) was the dominant mollusc. This snail was present in all ponds except 95.

Shelford Pond 52 (SP 52), much smaller in area than the younger ponds, had the same fauna as Pond 30 with the addition of *Promenetus exacuous* (Say, 1821) and the absence of *Physa gyrina* (Say, 1821), and *Musculium parteumeium*. This pond was in the last stage of the surface-reaching macrophyte association and was senescent. The characteristic mollusc was *Planorbella trivolvis*.

Gyraulus deflectus was absent from SP 93, leaving a depauperate molluscan community of six species: *Musculium securis*, *Musculium partumeium*, *Promenetus exacuous*, *Planorbella trivolvis*, *Planorbula armigera*, and *Stagnicola reflexa*. The southernmost pond sampled, SP 95, had only a single species of gastropod, *Planorbula armigera*, which is commonly found in intermittent habitats.

INDIANA DUNES

General background regarding the Indiana Dunes area is available in Cressey (1928), Daniel and Lerner (1984), Hill (1974), and Hill *et al.* (1991). Miller Woods (MW), a section of Indiana Dunes Lakeshore Park, is located at the western end of the city limits of Gary. The eastern boundary is Lake Street. Over 150 ponds occur in five recognizable rows running in an east-west direction and paralleling the southern shoreline of Lake Michigan (Fig. 3). The ponds are fed from north-flowing ground water (Wilcox and Simonin 1987). The youngest row, north of the Calumet River and closest to Lake Michigan has been designated MW Row 1. The most southerly and oldest row, farthest from Lake Michigan, is MW Row 5 (Wilcox *et al.* 1980). This numbering scheme has been followed by other researchers (Futyma 1985, Wilcox and Simonin 1987, Jackson *et al.* 1988) and is followed here.

Black mud surface sediment is absent from Row 1 ponds, but increases considerably from Row 2 to Row 5 (Table 1). The organic mud is underlain by mixtures of mud and sand with sand content increasing with depth. All evidence of marl has disappeared from the older ponds, correlating with a higher percentage of organics in these older ponds (Futyma 1985, Wilcox and Simonin 1987). All MW ponds have similar water chemistry. The pH values are between 7 and 8, conductivities between 320 and 429 µmhos/cm, and Ca^{++} values between 49 and 54 ppm. Nutrients tend to be low. Ammonium and organic nitrogen, however, increase from 0.25 in younger ponds to 0.43 in older ponds (Wilcox and Simonin 1987).

Ponds in MW Rows 1 and 2 are intradunal, formed during recession of Lake Michigan from the Algoma Stage to the modern elevation of 178 m (Reshkin 1981). Both are

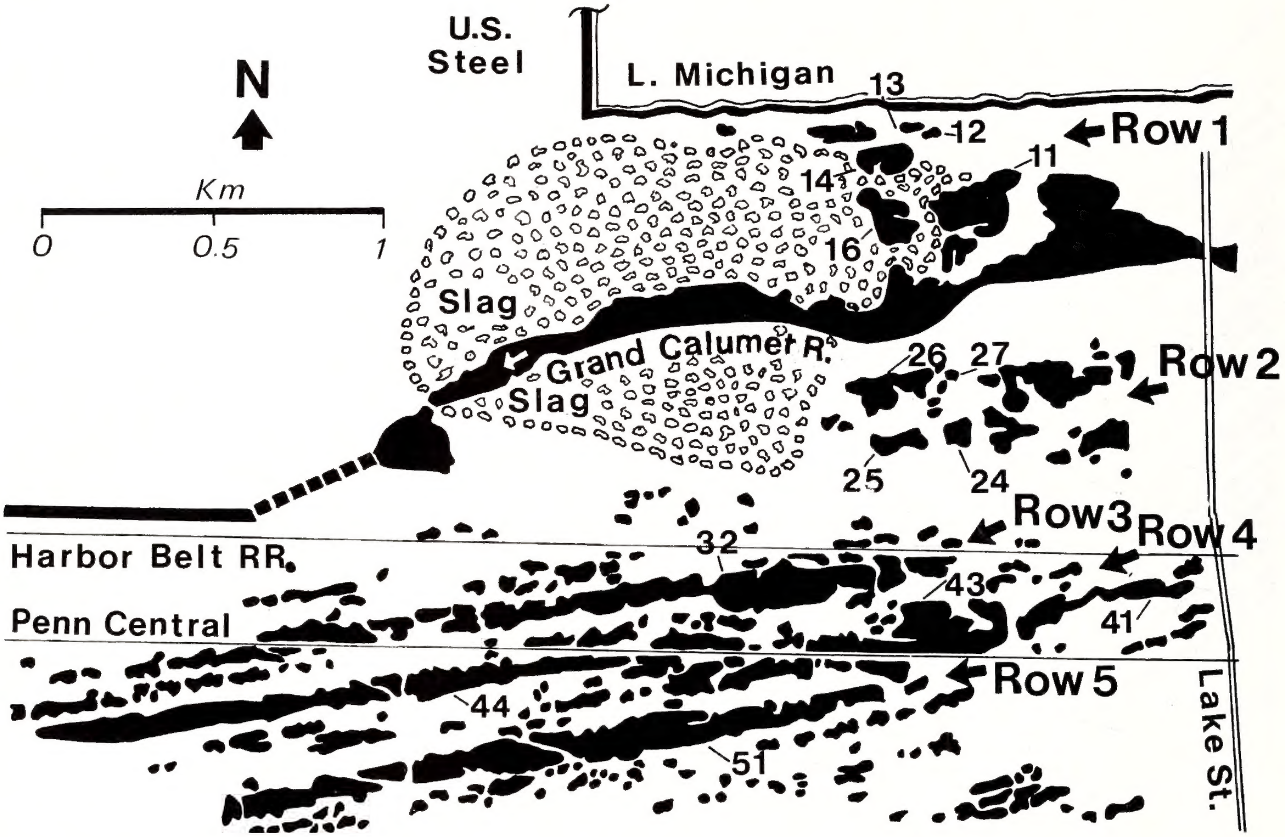

Figure 3. Map of the five rows of intra- and interdunal ponds in Miller Woods, Indiana Dunes National Lakeshore. Sampled ponds are numbered such that the first digit designates the row and the second digit the pond within the row (modified after Futyma 1985).

within a topography of hummocky dunes of irregular mounds cresting up to 21 m or more from the ponds' surfaces (Futyma 1985, Jackson *et al.* 1988). Slag was dumped in and around Row 1 ponds and extends west into the industrial area of Gary. Row 1 ponds have shallow, fine organic sediments mixed with individuals of *Chara* spp. and are underlain by nearly pure sand (Futyma 1985). Terrestrial vegetation on the dunes associated with Row 1 is early to mid-successional with scattered cottonwoods (illustrated in Daniel and Lerner 1984). Ponds in MW rows 1 and 2 were formed at the time Lake Michigan receded from the Algoma Stage to its present level (Reshkin 1981).

Ponds in MW Row 2 are similar in topography and in age to the ponds in MW Row 1 but are separated from them

Table 1. Physical and chemical features of the ponds at Miller Woods. Data from Futyma (1985) and Wilcox and Simonin (1987).

Row	Type	Age (year) BP	Elevation (m)	Distance from Lake Michigan (km)	Terrestrial vegetation	Aquatic vegetation	Water depth (cm)	Sediment depth (cm)	Mean pH	Sediment pH
1	Intradunal	200-300*	178	0.2-0.6	Dune grasses	*Chara* spp.	26.3	13.6	8	7.1
2	Intradunal	2100 ± 123	182.5-184	0.8-1.2	Black oak	*Chara* spp.	55.3	20.7	7.96	6.5
3	Interdunal	2100 ± 80	184.5	1.4	Black oak	*Myriophyllum* sp.	40.8	27	7.73	5.4
4	Interdunal	2400 ± 70	184.5	1.6	Black oak	*Myriophyllum* sp.	27.5	38.5	7.41	5.4
5	Interdunal	3000 ± 93	185.4	1.8	Black oak	*Typha* sp.	12.9	55.7	7.67	5.4

* dates estimated

by the west-flowing Grand Calumet River and a residential area. Five ponds in this row were numbered 21 through 25 (e.g., Row 2, fifth pond) (Futyma 1985). MW Ponds 26 and 27 are added here. The surrounding vegetation is a black oak savanna. The dominant aquatic vegetation for MW Rows 1 and 2 consisted primarily of *Chara vulgaris*, with the naiad *Najas flexilis* present but rare. In addition, ponds in Row 2 had individuals of great bladderwort and water milfoil (Futyma 1985, Wilcox and Simonin 1987). Slag was dumped to the west of these ponds and probably covered over other ponds.

MW Rows 3-5 are part of an interdunal ridge and swale formation. The ridges originated after 3000 BP (before present) during regression of Lake Michigan levels to the Algoma Stage. The ridges may be remnants of sand bars formed in shallow water offshore or rapidly formed parallel beach ridges (Reshkin 1981). The ridges are gently curved and crest 3-6 m above the ponds.

The aquatic flora of MW Rows 3-5 differed considerably from the *Chara* spp.-dominated ponds in Rows 1 and 2. *Chara* spp. were present but rare in Rows 3 and 4, where the dominant vegetation included water-milfoil, white water lily, dense stands of dead and living narrow-leaved cattail, and great bladderwort (Wilcox and Simonin 1987). The oldest pond, Pond 51, had standing water only in parts of the pond not choked with cattails. The most common plants, more marsh-like than those found in the younger ponds, consisted of spatterdock, bladderwort, mermaid-weed, water milfoil, marsh smartweed, and arrowhead. The terrestrial vegetation was a black oak savanna (Peattie 1930, Futyma 1985, Wilcox and Simonin 1987, and Jackson *et al.* 1988).

None of the ponds currently preserved within the Miller Woods section of Indiana Dunes Lakeshore Park adequately represents the oldest of Shelford's Ponds, which were intermittent in nature. Thus four habitats were sampled outside Miller Woods, two in Cowles Bog, 11 km east of West Beach, and two in ditches nearby (approximately 15 km east of the margin of Fig. 1). Cowles Bog is separated from West Beach by Midwest Steel, the Port of Indiana, Northern Indiana Public Service County, and Bethlehem Steel. The bog, formed as a lake 6200 BP, is a wetland complex of grassy fen, conifer swamp, and hardwood swamp. It lies 1 km south of Lake Michigan and is separated from the lake by a belt of dunes (Futyma 1985). The habitats sampled included the dry margin of a cattail marsh and a woodland vernal pool covered by terrestrial leaf litter.

One of the ditches sampled ran parallel to County Road 450 at 1300 North in the Heron Rookery, a section of the Park in Pine Township, Porter County. Terrestrial vegetation was wooded, and the dry substrate was sand overlain by terrestrial leaf litter. The second ditch, outside the Park in Chesterton, paralleled the Chesapeake and Ohio railroad tracks west of Route 49. The ditch was dry and filled with cattails.

MATERIALS AND METHODS

A total of 18 aquatic habitats within Indiana Dunes National Lakeshore Park and adjacent regions were sampled for molluscs, representing all five recognized pond rows as well as ditches, a vernal pool, and a dried marsh edge. A two-digit numbering scheme was adopted for the ponds, the first digit being the row number (Fig. 3). Collecting occurred during June and August of 1992 and June of 1993. Sampling was done by wading into the water and collecting floating and submerged plants and detritus with a dip net. Net contents were sorted for molluscs, and examination was made of terrestrial and dried detritus edging the water. Molluscs were narcotized with menthol crystals sprinkled into the collecting jars and left overnight. Once narcotized, the molluscs were fixed in buffered formalin for 24 hours and preserved in a solution of 70% ethanol and 5% glycerin. Specimens are housed at the field offices of Indiana Dunes National Lakeshore, 1100 North Mineral Springs Road, Porter, Indiana 46304.

The results were compared to lists compiled by Shelford (1913) from identifications made by Frank C. Baker at the University of Illinois. The more recent nomenclature of Burch (1989) and Clarke (1973, 1981) is followed here.

RESULTS

The molluscan species collected by Shelford (1913) from his 8 interdunal ponds are shown in Table 2. The results of the present survey of 14 ponds in the Miller Woods section of Indiana Dunes National Lakeshore, as well as of the four intermittent habitats combined, are shown in Table 3.

MW Pond 11 was unique among Row 1 ponds because it contained two species of "prosobranchs," the hydrobiid *Amnicola limosa* and the valvatid *Valvata tricarinata* (Say, 1817). "Prosobranchs" are gill-breathers and require relatively clean, well-oxygenated permanent bodies of water, such as lakes and rivers (Dillon 2000). The two pulmonates in Pond 11 were the physid *Physa gyrina* and the planorbid *Gyraulus parvus* (Say, 1817). These were common to almost all ponds in Rows 1 through 4. The sphaeriid clam *Pisidium casertanum* (Poli, 1795) was common to four of the five Row 1 ponds.

The other ponds in MW Row 1 contained only pulmonates. The typical pattern was one to two (usually one) lymnaeid, the physid *Physa gyrina*, and the planorbid *Gyraulus parvus*. Four species of sphaeriids, *Musculium lacustre* (Müller, 1774), *Musculium partumeium*, *Musculium securis*, and

Table 2. Molluscan species present (X) in interdunal ponds sampled by Shelford (1913). T = temporary; ? = species identification unsure.

Pond Number	1	5	7	14	30	52	93	95
Average depth (m)	0.3	0.5	0.5	0.4	0.2	0.1	T	T
Area (m^2 × 1000)	3.5	3.5	25	10	50	0.63	T	T
*Lampsilis siliquoidea**	X							
Anodonta grandis	X	X	X					
*Alasmidonta marginata**	X	X	X					
Musculium securis			X	?	X		X	
*Musculium partumeium**		X	X	X	X	X	X	
Amnicola limosa	X	X	X	X				
Cincinnatia cincinnatiensis	X	X	X	X				
Stagnicola reflexa	X	X	X	X	X	X	X	
Fossaria modicella	X	X	X					
Fossaria obrussa	X	X	X					
Physa gyrina	X	X	X	X	X			
Gyraulus parvus	X		X	X				
*Gyraulus deflectus**	X	X	X	X	X	X		
Helisoma anceps	X	X						
Planorbella campanulatum	X	X	X	X				
Planorbella trivolvis				X	X	X	X	
Promenetus exacuous						X	X	
Planorbula armigera				X	?	X	X	X
Number of species	13	12	13	11	7	6	6	1

* Species names updated from Clarke (1973): *Musculium partumeium* is a senior synonym of *Musculium truncatum; Gyraulus deflectus* is a senior synonym of *Gyraulus hirsutus* (Gould, 1840); *Lampsilis siliquoidea* is a senior synonym of *Lampsilis luteola* Simpson, 1914; and *Alasmidonta marginata* is transferred to the genus Anodonta Lamarck, 1799.

Pisidium casertanum, also inhabited Ponds 12-16, either together or separately. Diversity ranged from two to eight species of molluscs per pond. MW Pond 13 had the highest diversity due to four species of sphaeriids. Total species richness for MW Row 1 ponds combined was 10.

"Prosobranchs" were absent from ponds in MW Row 2. These ponds had similar faunas to those of MW Ponds 12-16 except for a decrease in sphaeriids to *Musculium securis*, the addition of *Planorbella trivolvis*, and the addition of two ancylids, *Ferrissia parallela* (Haldeman, 1841) and *Laevapex fuscus* (Adams, 1841). Also, in this row, all ponds contained the large stagnicoline lymnaeid, *Stagnicola reflexa* (Say, 1821), a species dominant in all remaining ponds. Diversity ranged from two species in the smallest pond (Pond 27) to six. Total species richness for MW Row 2 ponds combined was 8.

The only MW Row 3 pond sampled, pond 32, contained no sphaeriids and lacked any individuals of the amphibious lymnaeid genus *Fossaria* Westerlund, 1885.

The three ponds in MW Row 4 contained three species of sphaeriids, *Musculium lacustre*, *Musculium partumeium*, and *Musculium securis*. All ponds in Row 4 were similar in gastropod community structure to Pond 32 except for the ubiquity of *Ferrissia parallela* and the addition of two small planorbids, *Planorbula armigera* and *Promenetus exacuous*. Diversity was higher than in younger rows and ranged from six to eight molluscan species per pond. Total species richness for MW Row 4 was 10.

The molluscan fauna of MW Pond 51, the only Row 5 pond sampled, was generally similar to Row 4 ponds but without the two planorbids unique to Row 4.

The community composition for the four temporary habitats sampled was strikingly different from that of the intra- and interdunal ponds (Table 3). Two additional species of sphaeriids were collected, *Pisidium casertanum* and *Sphaerium occidentale* (Prime, 1853). The lymnaeids were represented by *Stagnicola elodes* (Say, 1821) and the physids by *Aplexa elongata* (Say, 1821). The planorbid was *Planorbula armigera*, also present in two ponds in MW Row 4. These species have the physiological ability to aestivate when their habitats dry (Jokinen 1978).

DISCUSSION

Industrial development over much of the area studied by Shelford (1913) makes modern comparison of his ponds with the Miller Woods ponds difficult (Whitman *et al.* 1988). However, generalized comparisons of the successional stages of both sets of ponds may be made. SP 1 had almost a clean sand substrate, SP 2 had mixed *Chara* spp., and SP 3 featured *Chara* spp. plus emergent and floating vegetation. The dominance displayed by submergent plants in MW Row 1 ponds (Wilcox and Simonin 1987, Magee 1981) suggests greatest similarity to SP 5. The numbers of sphaeriid and gastropod species richness were identical in SP 5 and in the five MW Row 1 ponds (combined), but the molluscan communities differed (Tables 2-3). Compared to SP 5, MW Row 1 ponds had three additional species of sphaeriid, one additional lymnaeid, two fewer planorbids, one less hydrobiid, and a valvatid. The most obvious difference was the absence of the planorbids *Helisoma anceps* and

Table 3. Molluscan species present (X) in intra- and interdunal ponds at Miller Woods and in temporary aquatic habitats (T) in Cowles Bog, the Heron Rookery, and a railroad ditch outside the park (combined).

Pond Number	11	12	13	14	16	24	25	26	27	32	41	43	44	51	T
Age of habitat (BP)	2000					2000				2100	2400			3000	
Musculium lacustre			X									X			
Musculium partumeium	X		X								X	X	X		X
Musculium securis			X			X					X			X	
Pisidium casertanum	X	X	X		X										X
Sphaerium occidentale															X
Valvata tricarinata	X														
Amnicola limosa	X														
Fossaria sp.			X	X	X	X	X	X							
Stagnicola reflexa		X	X	X		X	X	X	X	X	X	X	X	X	
Stagnicola elodes															X
Physa gyrina	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Aplexa elongata															X
Gyraulus parvus	X	X	X		X	X	X			X	X	X			
Planorbella trivolvis						X	X	X		X	X		X	X	
Planorbula armigera												X	X		X
Promenetus exacuous												X			
Ferrissia parallela								X			X	X	X	X	
Laevapex fuscus								X							
Number of species	6	4	8	3	4	6	5	6	2	4	7	8	6	4	6

Planorbella campanulatum in Miller Woods. In fact, although these species were common in Shelford's newer ponds, neither species seemed to occur in any MW pond sampled. Although *Amnicola limosa* lived in both sets of ponds, SP 5 had *Cincinnatia cincinnatiensis*, but MW row 1 had a population of *Valvata tricarinata*. All three "prosobranchs" are usually in lakes and larger rivers (Jokinen 1992), and they may represent a fauna having its origin in Lake Michigan or the Grand Calumet River.

A comparison of Shelford's results for eight interdunal ponds with the 14 MW ponds demonstrates three specific distribution patterns: (1) Ubiquitous species (found across all ages of ponds, but not necessarily every pond): *Musculium securis*, *Physa gyrina*, and *Stagnicola reflexa*; (2) Species that occur in younger ponds, with overlap into mature ponds (SP 1, 5, 7, 14; MW rows 1 and 2): *Amnicola limosa* and *Fossaria* sp.; (3) Species that occur in mature to older ponds that are becoming marshes or swamps (SP 14, 30, 52, 93, and 95; MW Rows 3, 4, and 5): *Promenetus exacuous*, *Planorbula armigera*, and *Planorbella trivolvis*. Two species demonstrated opposite patterns between the two pond sets: *Musculium partumeium* and *Gyraulus parvus* were ubiquitous in MW ponds, but *M. partumeium* was only found in Shelford's older ponds, and *G. parvus* was only in Shelford's younger ponds.

In addition to the above species, other groups were present in one set of ponds but absent from the other. Species present in MW but absent in SP were: *Musculium lacustre*, *Pisidium casertanum*, *Valvata tricarinata*, *Ferrissia parallela*, and *Laevapex fuscus*. The latter two species, both limpets, are common in ponds with emergent and floating-leaved vegetation with allochthonous organics typical of senescent ponds that are in the process of becoming marshes (Jokinen 1992 and references therein). It is puzzling that they were absent from all of Shelford's ponds. Species present in Shelford's ponds but absent in MW ponds were: *Gyraulus deflectus*, *Cincinnatia cincinnatiensis*, *Helisoma anceps*, and *Planorbella campanulatum*. *Cincinnatia cincinnatiensis* is typical of habitats in lakes and slow rivers with little vegetation (Berry 1943); the population probably represented a stranded population from Lake Michigan.

The four temporary habitats examined in the present survey demonstrated a unique molluscan community structure (Table 3). Ruthven (1911) found similar community composition between permanent and temporary ponds of the sand dune region of Saginaw Bay (Lake Huron). The same community structure also occurs in temporary habitats in southern Michigan, Connecticut, and New York (Jokinen 1992 and pers. obs.).

A comparison of overall successional pattern in Shelford's Ponds of 1913 and in the Indiana Dunes environment of today leaves a first impression of striking contrast. Tables 2 and 3 list 18 molluscan species, but only 10 (or perhaps 11) are shared (depending on the identity of the Miller Wood species of *Fossaria*). Shelford's Ponds were inhabited by three unionid species, three planorbids, and a hydrobiid not found in the present study of the Indiana Dunes area, while the Indiana Dunes ponds yielded three sphaeriids, two ancylids, a lymnaeid, and a physid not reported by Shelford. The impression left by these data is one of community volatility, suggesting that nondeterministic factors control the structure of the molluscan assemblage.

A second impression is made by a comparison of the overall species richness in Tables 2 and 3. The first four of Shelford's Ponds included 11-13 molluscan species, but no pond in the current fauna of Millers Woods supported more than 8. Although this difference may reflect the generally greater surface area of Shelford's lower-numbered ponds, it is also possible that environmental degradation is at least partially responsible.

Despite the large overall differences between the 1913 fauna and the fauna of today, a careful comparison of Tables 2 and 3 reveals several striking similarities. The three planorbids shared by the two data sets, *Planorbula armigera*, *Promentus exacuous*, and *Planorbella trivolvis*, were absent from the younger, larger ponds and generally present in the smaller, older ones, while the reverse was true of the species of *Fossaria*. Dividing Shelford's ponds into a younger set of three and an older set of five, there are 11/15 occurrences of *P. armigera*, *P. exacuous*, and *P. trivolvis* in the latter but 0/9 in the former. Similarly dividing the modern data into a set of five young ponds and ten older habitats, the occurrences are 0/15 in the former and 11/30 in the latter. This does not give the appearance of chance.

Planorbella (or *Helisoma*) *trivolvis* was one of the two species of freshwater gastropods upon which Dillon (2000) based his concept of R-adaptation. Such species, characterized by a reproductive effort greater than expected for their body size by an order of magnitude, are rapidly-maturing, likely to reproduce asexually, and semelparous. Dillon suggested that these life history specializations will permit R-selected species to colonize habitats that are rich but unpredictable, such as shallow and ephemeral ponds in late successional stages. It seems likely that all three of the planorbids shared by Tables 2 and 3 have benefited from R-adaptation.

Dillon (2000) concluded that freshwater molluscan communities are best described as "minimally structured." He suggested that the specific composition and relative abundance of typical freshwater gastropod faunas will largely be a function of nondeterministic factors, but that deterministic effects (such as specific adaptations by some elements of the fauna to differing successional stages) generally result in communities that are not entirely random collections of species. Such a model fits the pattern of molluscan community succession in ponds of the Indiana Dunes National Lakeshore quite well.

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Glochidial morphology of selected species of the genera *Cristaria* Schumacher, 1817 and *Sinanodonta* Modell, 1945 (Bivalvia: Unionidae) from Far Eastern Russia

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Abstract: Light and scanning electron microscopy (SEM) revealed similarities and differences in the glochidia of four species belonging to two unionid genera from Primorye Territory in Far Eastern Russia: *Cristaria herculea, Cristaria tuberculata, Sinanodonta amurensis,* and *Sinanodonta likharevi*. Glochidia of the two species of *Cristaria* Schumacher, 1817 differed from each other in morphological details of the hook (hook length, maximum height of hook microstylets) and hinge, but not in shape (height/length ratio) or size. Glochidia of the two species of *Sinanodonta* Modell, 1945 differed from each other in quantitative features such as height, length of hinge, and shape (height/length ratio). Glochidia of these two species of *Sinanodonta* lacked some characters possessed by other genera in the tribe Anodontini, in which *Sinanodonta* has been classified, and shared some characters with the two species of *Cristaria,* which are classified in the tribe Limnoscaphini, raising uncertainty about the correct classification of *Sinanodonta.*

Key words: Anodontini, *Cristaria herculea, Cristaria tuberculata,* Glochidia, Limnoscaphini, *Sinanodonta amurensis, Sinanodonta likharevi*

The adult shells of many unionid bivalves provide little information for reliable identification at the specific, generic, or even subfamilial level. The larvae (glochidia), however, are highly specialized because of their complex lifecycles and can provide characters that are useful for distinguishing among species and higher taxa. The shape, size, dentition, exterior sculpturing, and pit size of the glochidia all contribute useful taxonomic information (Hoggarth 1999).

There is disagreement about the number of species in the genus *Cristaria* Schumacher, 1817 (tribe Limnoscaphini Lindholm, 1932). Different authors include three (Haas 1969), four (Inaba 1964), or six (Moskvicheva 1973) species. Although Haas (1969) and Brandt (1974) considered both *Cristaria herculea* (Middendorff, 1847) and *Cristaria tuberculata* Schumacher, 1817 to be junior synonyms of *Cristaria plicata* (Leach, 1815), Russian malacologists have recognized *C. herculea* and *C. tuberculata* as full species for many years (Zatravkin and Bogatov 1987).

Forms identified as *Cristaria herculea* and *Cristaria tuberculata* inhabit the Amur River basin and Khanka Lake in eastern Russia. Shadin (1938) made the first short note on the glochidium of *C. herculea.* Antonova and Starobogatov (1988) reported on the glochidium of *C. tuberculata,* but their illustrations lack sufficient detail to be useful and their text measurements differ from those in the illustrations.

We studied the glochidia of *Cristaria herculea* and *Cristaria tuberculata* to see if glochidial characters could provide useful characters for addressing the species status of these two *Cristaria* species.

The species-rich Asian genus *Sinanodonta* Modell, 1944 has traditionally been classified in the tribe Anodontini Rafinesque, 1820, which also includes *Anemina*-group bivalves in the genera *Anemina* Haas, 1969, *Amuranodonta* Moskvicheva, 1973, and *Buldowskia* Moskvicheva, 1973. Shadin (1938) gave a preliminary description of the glochidium of *Sinanodonta woodiana* (Lea, 1834) (as *Anodonta woodiana*) without any illustrations. Inaba (1941, 1964) gave descriptions and schematic illustrations using light microscopy of the glochidia of *S. woodiana* (as *A. woodiana lauta* Martens, 1877 and *A. woodiana lauta tumens* Haas, 1910) and *Sinanodonta calipygos* (Kobelt, 1879) (as *A. woodiana calipygos*). Antonova and Starobogatov (1988) illustrated the glochidia of bivalves they tentatively identified as *Sinanodonta amurensis* Moskvicheva, 1973 using light microscopy, but again, their text measurements differed from those in the illustrations. Scanning electron microscopic studies on glochidia of *S. woodiana* (as *A. woodiana*) from Korea focused on the fine structure of the glochidia (Jeong 1989, Lee *et al.* 1989, Jeong *et al.* 1993, Kwon *et al.* 1993, Park and Kwon 1993). These studies compared the glochidia of species of *Sinanodonta* with those of species of *Anemina* and documented that glochidial features vary. Further examination of the glochidia of members of the genus *Sinanodonta*, especially of species that have not been examined previously, would improve our knowledge of this group of bivalves.

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MATERIALS AND METHODS

Source and deposition of specimens

Glochidia were obtained from gravid females of *Cristaria herculea* (Primorye, Khanka Lake near Kamen'-Rybolov village; n = 1; 10 October 1996; collectors T.S. Vshivkova, T.V. Nikulina); *C. tuberculata* (Primorye, Khanka Lake near Vostochnyi kordon; n = 1; 28 October 1999; collector L.A. Prozorova); *Sinanodonta amurensis* (Primorye, Razdolnaya River; n = 1; 21 June 1999; collector L.A. Prozorova), and *S. likharevi* Moskvicheva, 1973 (Primorye, Ilistaya River basin near Sibirtsevo village; n = 1; 19 May 1996; collector V.A. Dvoryadkin). Sample sizes of adults were small because it was very difficult to find gravid females with mature glochidia. We assumed the developmental stages of all glochidia were the same across the species and could be compared directly because female unionids of the subfamily Anodontinae hold mature glochidia in their gills all winter, during which time the glochidia do not change appreciably in size (Shadin 1938, Antonova 1991, Chernyshev 1998).

Specimens of adult bivalves and glochidial samples were deposited at the Russian Academy of Sciences, Far Eastern Branch, Institute of Biology and Soil Sciences, Vladivostok, Russia. Samples of glochidia of three of the species were also deposited at the Delaware Museum of Natural History, Wilmington, Delaware, USA: *Cristaria herculea* (DMNH 221699), *Cristaria tuberculata* (DMNH 221700), and *Sinanodonta amurensis* (DMNH 221701).

Examining glochidia

Mature glochidia were recovered from the demibranchs of gravid females and fixed in 75% ethanol for investigation with both light and scanning electron microscopy (SEM). To extract the specimens from the gill tissue, we washed ethanol-fixed glochidia in three changes of distilled water, cleaned them in 5% KOH for 1.5-2 hours, and washed them at least five times with distilled water (following Kwon *et al.* 1993).

Measurements to be used in statistical comparisons were made using light microscopy on at least 20 glochidia per adult female, but not all measurements could be made on every glochidium. Both temporary water-glycerin and permanent glycerin-gelatin preparations were used for this purpose. We measured valve length parallel to the hinge (length) (n = 20-27 per species), valve height perpendicular to the hinge (height) (n = 15-22), length of hinge (hinge) (n = 20-22), and length of the hook (hook) (n = 5-19; no measurements of hook were made on *Sinanodonta likharevi*). We made these measurements using an Olympus light microscope fitted with an ocular reticle. For terminology of glochidia see Kondo and Yamashita (1980); regarding anterior-posterior orientation of glochidia see Hoggarth (1987). To standardize the measurements, we calculated three ratios: height/length, hinge/length, and hook/height.

Morphological details of the hooks and pits of the glochidia of *Cristaria herculea, Cristaria tuberculata,* and *Sinanodonta amurensis* were examined using SEM. The SEM details were used for qualitative descriptions. Glochidia of *Sinanodonta likharevi* were not available for SEM work. We made one SEM photomicrograph for each of seven views for each of the three species, sometimes photographing a different glochidium on the SEM stub for different views. We made images of glochidium shape, hinge, pits on the inner valve surface, pits on the outer valve surface, hook viewed perpendicular to the plane of the valve commissure, close-up of hook base, and hook in side view.

To prepare specimens for SEM, we pipetted glochidia to a 2-ml polyethylene centrifuge tube and shook them vigorously in distilled water for 30 seconds to separate soft parts from valves (Kinzelbach and Nagel 1986, Kwon *et al.* 1993). After the glochidial valves settled for 20-30 seconds, the liquid containing suspended soft parts was siphoned with a pipette. The shaking, settling, and siphoning procedure was repeated twice with distilled water; we used 95% ethanol in place of distilled water in the fourth and final iteration to hasten glochidia desiccation. Moist glochidia were removed from the centrifuge tube with a fine wooden pick and placed on double-sided carbon tape on a SEM stub. Using a Denton Vacuum Desk II sputter coater (Cherry Hill, New Jersey) we coated the glochidia with gold-palladium about 100 Å thick. A Topcon ABT-60 Scanning Electron Microscope (Topcon Technologies, Inc., 69406 Koll Center Parkway, Pleasanton, California 94566, USA) with a 10 kV electron beam was used to view the glochidia.

Intra- *vs.* interspecific glochidial variation: Glochidia from one female per species

We examined multiple glochidia from a single female from each species because additional gravid females were unavailable. However, if multiple glochidia from a single female do not adequately represent glochidial variation from the whole species, then interspecific comparisons using characters of those glochidia could give misleading conclusions. Furthermore, if variation of glochidia from different females of a single species were greater than the variation among females of different species, then glochidial features would not be reliable for discriminating among species. To address these concerns, we examined a published dataset to compare intraspecific variation in glochidia to interspecific variation to test whether glochidia from a single female could be used for discriminating among species.

We used data from Hoggarth (1999, table 2) to compare the morphological variation of glochidia within a single female to that between females of the same species and to the variation of glochidia among females of different species. We included species from the Anodontinae only because our species are members of that group. For 10 species in the Anodontinae, Hoggarth had measured glochidial characters from more than one female per species (with at least 2 glochidia per female). The species and the numbers of glochidia measured from each of two females are *Anodonta anatina* (Linnaeus, 1758) (5 glochidia, 2 glochidia), *Anodonta kennerlyi* Lea, 1860 (4, 2), *Pyganodon grandis grandis* (Say, 1829) (4, 3), *Pyganodon cataracta cataracta* (Say, 1817) (3, 2), *Utterbackia imbecillis* (Say, 1829) (8, 3), *Anodontoides ferussacianus* (Lea, 1834) (3, 4), *Lasmigona compressa* (Lea, 1829) (2, 2), *Lasmigona subviridis* (Conrad, 1835) (3, 2), *Lasmigona holstonia* (Lea, 1838) (3, 2), and *Lasmigona costata* (Rafinesque, 1820) (4, 3). We compared inter-female to inter-species variation for glochidial measurements of length, height, and hinge length using ANOVA for those 10 species.

While glochidia measurements differed significantly among species (length, height, hinge length all $p < 0.0001$), they did not differ significantly among females (length, $p = 0.454$; height, $p = 0.745$; hinge length, $p = 0.212$).

The results of this analysis of data from Hoggarth (1999) found greater interspecific than intraspecific variation in glochidia for these 10 species of Anodontinae. Furthermore, glochidia did not differ significantly between females of the same species. Using characters of the glochidia, we were not likely to misclassify conspecific females of these 10 species as different species.

If we could extrapolate this result to members of the Anodontinae from Far Eastern Russia, we should be able to determine whether single individual females are members of the same species by comparing their glochidia. In contrast, some Far Eastern Russian species of Anodontinae in the *Anemina* group (*Anemina* spp., *Amuranodonta* spp., *Buldowskia* spp.) do show large variation in their glochidial measurements among populations or even among individual females from the same population (Sayenko 1999b, 2000, 2003). However, species of other Far Eastern Russian anodonine genera that are more similar to species of *Cristaria,* including *Beringiana* Starobogatov in Zatravkin, 1983, do not show much intraspecific variation (Sayenko *et al.* 2001). Detailed investigations have not yet been made for glochidia of species in the genera *Cristaria* and *Sinanodonta,* but because these genera appear to be more closely related to *Beringiana* than to species in the *Anemina* group, we expect that their glochidia would not show much intraspecific variation. Therefore, finding statistically significant differences in glochidia from different females of *Cristaria* spp. and *Sinanodonta* spp. would be evidence supporting the idea that the females belong to different species.

Data analysis

For each species, we performed single-factor analysis of variance (ANOVA) on each glochidial character using SYSTAT 10 (SPSS Inc., 233 South Wacker Drive, 11th floor, Chicago, Illinois, 60606, USA). We used the least significance difference (LSD) post-hoc test to evaluate significance among the four species.

RESULTS

Cristaria species

Cristaria herculea (Figs. 1A, 2A, 3A-B, 4A-B, 5). The valves of the glochidia of *C. herculea* were distinctly asymmetric because the ventral (hooked) edge of each valve was displaced posteriorly and because the anterior edge was longer and had a more prominent curve (Fig. 1A). Valve length (parallel to hinge) ranged from 271-285 μm (mean 274.2, st. dev. 4.1 μm) and valve height (perpendicular to hinge) ranged from 271-293 μm (mean 281.2, st. dev. 4.9 μm). Valve height was greater than valve length with the height/length ratio ranging from 1.01-1.05 (mean 1.032, st. dev. 0.013). Hinge length (Fig. 2A) was 193-207 μm (mean 199.9, st. dev. 4.5 μm), being 70-76% of valve length (mean 73.0, st. dev. 1.5%). The hook ranged from 100-114 μm (mean 106.2, st. dev. 4.9 μm), being from 36-41% of the valve height (mean 37.7, st. dev. 1.8%). The glochidium examined by SEM had approximately 20 large microstylets arranged on the hook in 2-3 poorly defined longitudinal rows (Fig. 3A-B). Maximum height of the microstylets on the hook was 12.7 μm. The pits on the inner valve surface (0.5-0.6 μm diameter) tended to be smaller than those on the outer surface (0.5-1.2 μm diameter) (Figs. 4A-B) and pits were situated on the entire valve including the adductor muscle attachment site.

Cristaria tuberculata (Figs. 2B, 3C-D, 4C-D, 5). The valves of *C. tuberculata* glochidia were distinctly asymmetric because the ventral (hooked) edge of each valve was displaced posteriorly and because the anterior edge was longer and had a more prominent curve (Fig. 2B). Valve length (parallel to hinge) ranged from 270-286 μm (mean 276.7, st. dev. 6.3 μm) and valve height (perpendicular to hinge) ranged from 271-300 μm (mean 284.4, st. dev. 7.5 μm). Valve height was greater than valve length with the height/length ratio ranging from 1.03-1.06 (mean 1.036, st. dev. 0.010). Hinge length (Fig. 2B) was 207-221 μm (mean 213.5, st. dev. 5.5 μm), being 75-80% of valve length (mean 77.0, st.

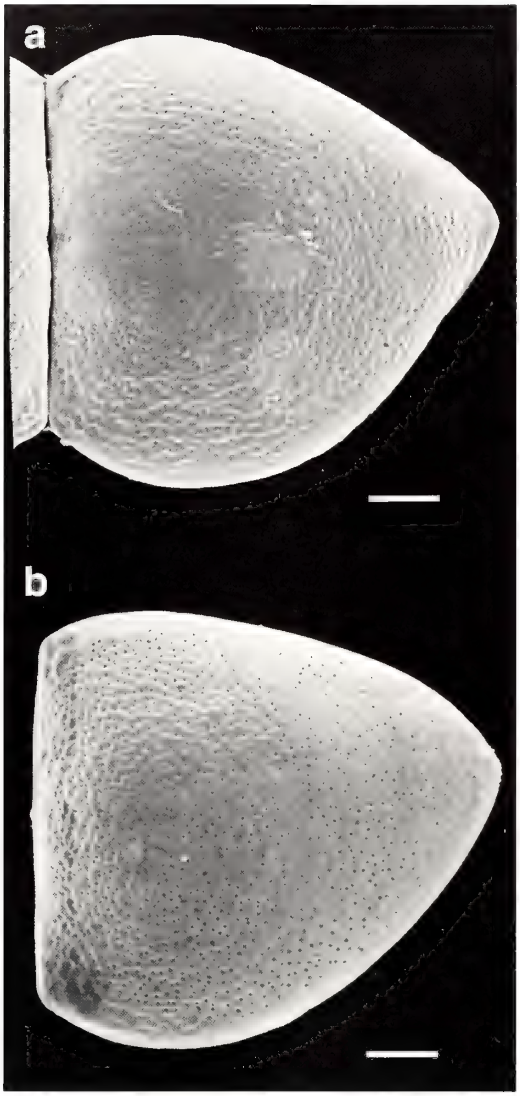

Figure 1. Scanning electron micrographs showing the shapes of the valves of the glochidia of A, *Cristaria herculea,* scale bar = 35 μm and B, *Sinanodonta amurensis,* scale bar = 40 μm.

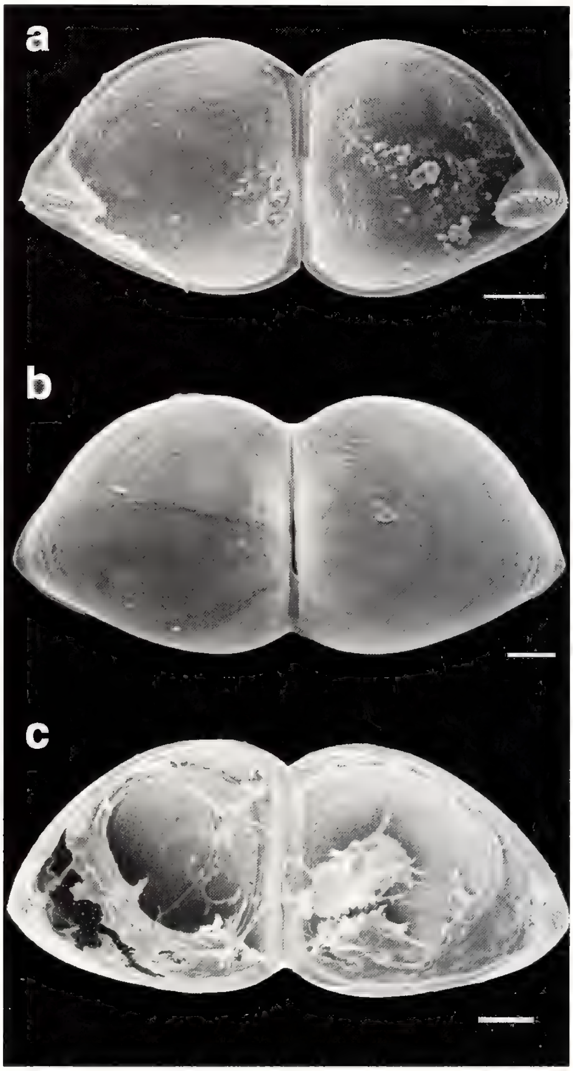

Figure 2. Scanning electron micrographs illustrating the hinge lengths of the glochidia of A, *Cristaria herculea* (internal view); B, *Cristaria tuberculata* (external view); and C, *Sinanodonta amurensis* (internal view). All scale bars = 50 μm.

dev. 1.7%). The hook ranged from 106-114 μm (mean 111.3, st. dev. 3.4 μm), being from 38-42% of the valve height (mean 39.9, st. dev. 1.8%). The glochidium examined by SEM had about 17 large microstylets arranged on the hook in 1-2 poorly defined longitudinal rows (Fig. 3C-D). Maximum height of the microstylets on the hook was 9.1 μm. The pits on the inner surface (0.5-1.2 μm diameter) tended to be slightly smaller than those on the outer surface (0.6-1.5 μm diameter) (Fig. 4C-D), although there was considerable overlap in pit diameters. Pits were situated on the entire valve including the site of adductor muscle attachment.

Similarities in the glochidia of *Cristaria herculea* and *Cristaria tuberculata* included valve shapes, both species having distinctly asymmetric valves with the anterior edge longer and with a more prominent curve (Figs. 1A, 2B). Glochidial valves of the two species did not differ significantly in size or shape (LSD post hoc tests after ANOVA for length, $p = 0.209$; height, $p = 0.131$; height/length, $p = 0.535$) (Fig. 5A-C). In one glochidium measured of each species there was overlap in the pit diameters on the internal surfaces of the glochidial valves of the two species (0.5-0.6 versus 0.5-1.2 μm, *C. herculea* and *C. tuberculata,* respectively)

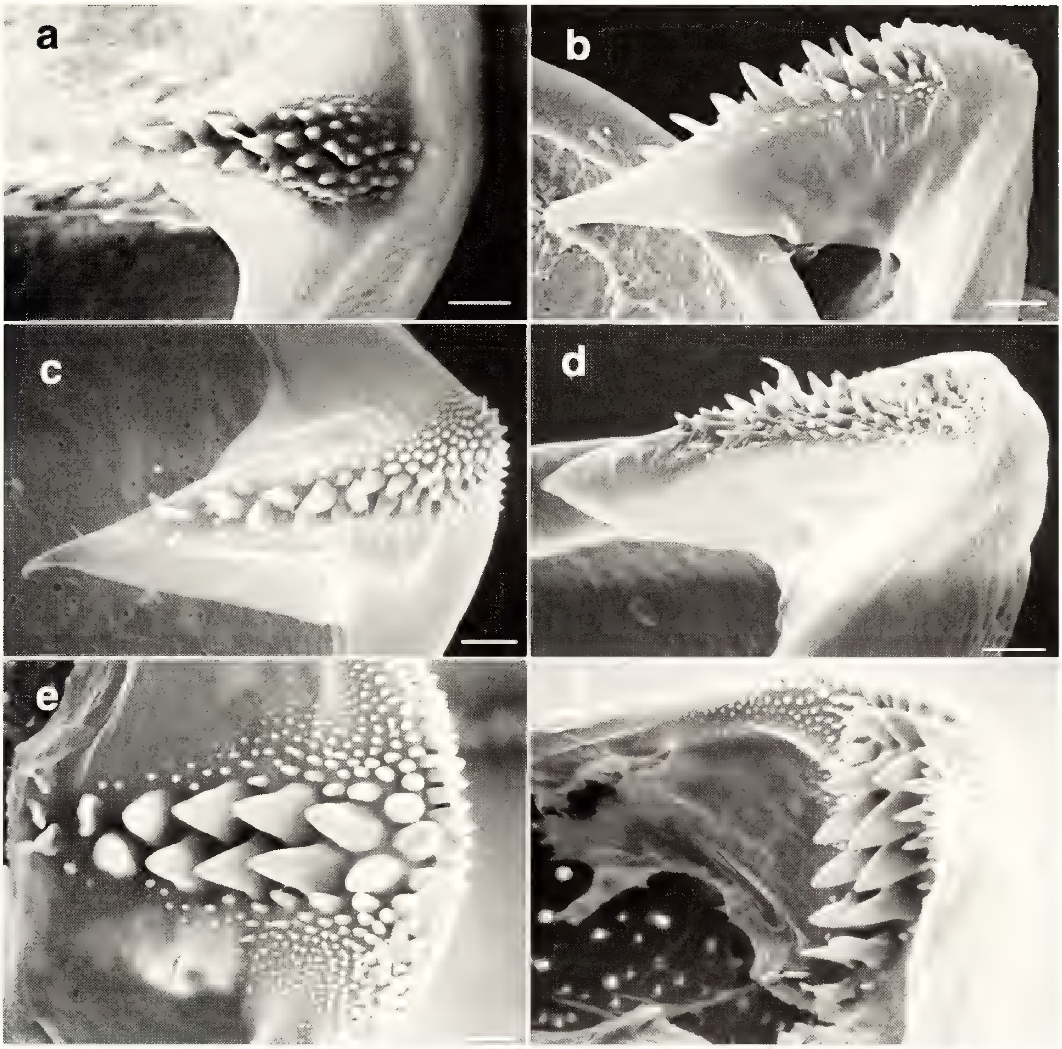

Figure 3. Scanning electron micrographs showing the hooks of the glochidia of A-B, *Cristaria herculea;* C-D, *Cristaria tuberculata;* E-F, *Sinanodonta amurensis.* Figures A, C, and E show the hook viewed perpendicular to the plane of the valve commissure. Figures B, D, and F show the hook in side view. All scale bars = 10 μm.

and on the external surfaces (0.5-1.2 versus 0.6-1.5 μm, respectively) (Fig. 4A-D).

Glochidia of the two species differed significantly in hinge and hook characters. Glochidia of *Cristaria tuberculata* had longer absolute and relative hinge lengths and longer absolute and relative hook lengths (LSD post hoc tests for hinge, p = 0.000; hinge/length, p = 0.000; hook, p = 0.000; hook/height, p = 0.001) (Fig. 5D-G). In one glochidium measured of each species, maximum height of the microstylets on the hook was greater in *Cristaria herculea* (12.7 μm) than in *C. tuberculata* (9.1 μm) (Fig. 3B,D). There were more small microstylets at the flex point of the hook in *C. tuberculata* than in *C. herculea* (Fig. 3A,C). Pits on the inner surfaces of the valves tended to be slightly smaller than those on the outer surfaces for both species of *Cristaria* (Fig. 4A-D).

Sinanodonta species

Sinanodonta amurensis (Figs. 1B, 2C, 3E-F, 4E-F, 5). The valves of the glochidia of *S. amurensis* were distinctly asymmetric because the ventral (hooked) edge of each valve was displaced posteriorly and because the anterior edge was longer and had a more prominent curve (Fig. 1B). Valve length (parallel to hinge) ranged from 254-264 μm (mean 258.6, st. dev. 3.7 μm) and valve height (perpendicular to hinge) ranged from 257-278 μm (mean 270.6, st. dev. 5.8

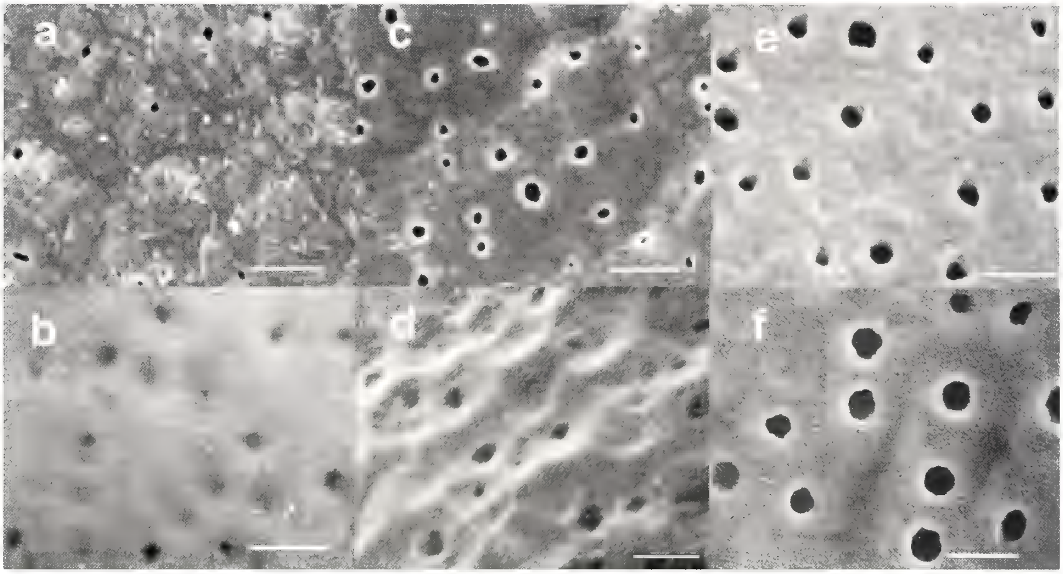

Figure 4. Scanning electron micrographs showing pits on the surfaces of the glochidial valves of A-B, *Cristaria herculea;* C-D, *Cristaria tuberculata;* E-F, *Sinanodonta amurensis.* Figures A, C, and E (top row) are of the internal valve surfaces. Figures B, D, and F (bottom row) are of the external valve surfaces. All scale bars = 5μm.

μm). Valve height was greater than or equal to valve length with the height/length ratio ranging from 1.00-1.07 (mean 1.041, st. dev. 0.020). Hinge length (Fig. 2C) was 186-214 μm (mean 202.7, st. dev. 6.8 μm), being 76-81% of valve length (mean 78.9, st. dev. 2.4%). The hook ranged from 96-100 μm (mean 99.2, st. dev. 1.6 μm), being from 36-38% of the valve height (mean 37.1, st. dev. 1.0%). The distorted appearance of the hook of *S. amurensis* in the SEM images (Fig. 3E-F) is an artifact of drying for SEM. Hooks were not distorted in wet specimens of this species that were measured for ANOVA. The glochidium examined by SEM had about 12 large microstylets arranged on the hook in two well-defined longitudinal rows (Fig. 3E-F). Maximum height of the microstylets on the hook was 13.6 μm. The pits on the inner surface (0.8-1.8 μm diameter) were smaller than those on the outer surface (1.6-2.0 μm diameter) (Fig. 4E-F). Pits were situated on the entire valve including the site of adductor muscle attachment.

Sinanodonta likharevi (Fig. 5). The valves of the glochidia of *S. likharevi* were distinctly asymmetric because the ventral (hooked) edge of each valve was displaced posteriorly and because the anterior edge was longer and had a more prominent curve. Valve length (parallel to hinge) ranged from 250-293 μm (mean 259.6, st. dev. 9.8 μm) and valve height (perpendicular to hinge) ranged from 264-314 μm (mean 283.6, st. dev. 10.9 μm). Valve height was greater than valve length with the height/length ratio ranging from 1.03-1.14 (mean 1.094, st. dev. 0.033). Hinge length was 171-218 μm (mean 194.4, st. dev. 11.0 μm), being 65-86% of valve length (mean 75.0, st. dev. 3.8%). We were unable to measure hook lengths of the glochidia of *S. likharevi* by light microscopy or to examine microstylets or pits by SEM because the hooks of the glochidia of *S. likharevi* were damaged.

Glochidial valves of *Sinanodonta amurensis* and *Sinanodonta likharevi* did not differ significantly in length (LSD post hoc test for length, p = 0.654) (Fig. 5A). The glochidia of *S. amurensis* and *S. likharevi* differed significantly in height and hinge length. The glochidia of *S. amurensis* were smaller in absolute and relative height (Fig. 5B-C) and larger in absolute and relative hinge length (LSD post hoc test for height, p = 0.000; height/length, p = 0.000; hinge, p = 0.002; hinge/length, p = 0.000).

Comparisons between *Cristaria* and *Sinanodonta*

ANOVA of the seven glochidial characters of the four species showed significant variation in all seven characters (Table 1). Letters in Fig. 5 indicate which species differ significantly in which characters from each other, as determined by LSD post-hoc tests.

There were a number of similarities in glochidia of the two species of *Cristaria* and the two species of *Sinanodonta.* In qualitative characters, glochidia of both genera were similarly asymmetric with the ventral (hooked) edge of each valve displaced posteriorly and with the anterior edge longer

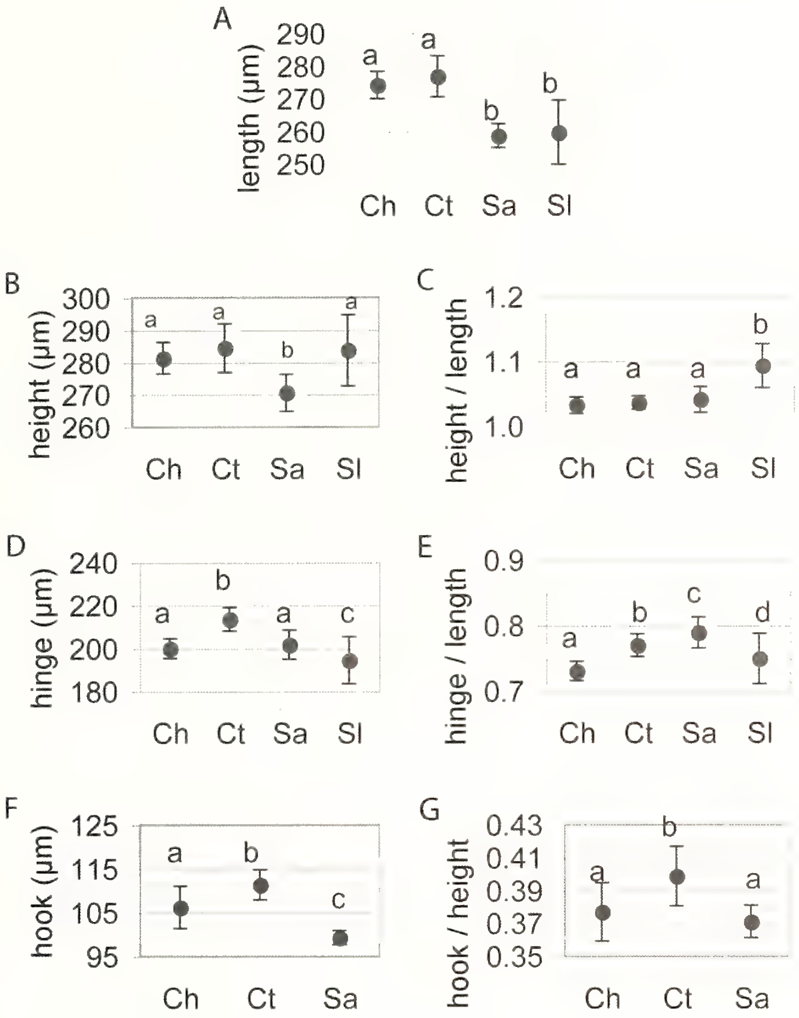

Figure 5. Mean values of seven characters of glochidia of *Cristaria herculea* (Ch), *Cristaria tuberculata* (Ct), *Sinanodonta amurensis* (Sa), and *Sinanodonta likharevi* (Sl) (five characters). Length is measured parallel to the hinge. Error bars are standard deviation. Different letters indicate values that differ significantly at $p < 0.05$.

and with a more prominent curve (Figs. 1-2). The pits on the inner surfaces of the glochidia of the two species of *Cristaria* and *Sinanodonta amurensis* tended to be smaller than those on the outer surfaces (Fig. 4) and pits were situated on the entire valve including the site of adductor muscle attachment.

Five quantitative characters (height, relative height, hinge length, relative hinge length, and relative hook length) varied from being significantly to not significantly different among the species, but they did not differ consistently between genera. Glochidial heights of the two species of *Sinanodonta,* ranging from 257-314 µm, completely overlapped the heights of the two species of *Cristaria* (271-300 µm) (Fig. 5B) and in both genera, the relative heights were always 1.00 or greater (1.00-1.14 height/length) (Fig. 5C). Glochidial hinge length overlapped considerably between the two genera (Fig. 5D), with the two species of *Cristaria* ranging from 193-221 µm, and the two species of *Sinanodonta* ranging from 171-218 µm. While the relative hinge lengths (standardized by valve length) of all four species differed significantly (LSD post hoc p-values ranged from $p = 0.000$ to $p = 0.031$), the direction of the difference was not consistent between genera (Fig. 5E). Relative hook lengths of the two species of *Cristaria* (36-42% of shell height) overlapped those of *Sinanodonta amurensis* (36-38% of shell height) (Fig. 5G).

On the other hand, there were some intergeneric differences in glochidia. The only quantitative character that differed significantly between genera but not within genera was length, with glochidia of *Cristaria* spp. being longer than those of *Sinanodonta* spp. (Fig. 5A) (species of *Cristaria* ranged 270-286 µm in length, species of *Sinanodonta* ranged 250-268 µm in length except one unusually large individual glochidium of *Sinanodonta likharevi* that was 293 µm long). Glochidial hooks in both species of *Cristaria* (hooks 100-114 µm long) were significantly longer than those of *Sinanodonta amurensis* (96-100 µm long) (LSD post hoc test after ANOVA for hook between *S. amurensis* and *Cristaria herculea,* $p = 0.002$; between *S. amurensis* and *Cristaria tuberculata,* $p = 0.000$) (Fig. 5F).

In characters examined on a single glochidium of each species, glochidial differences between the two genera included the position of the hook, valve curvature near the hinge, pit size, and arrangement and morphology of the microstylets on the hook. The hook area in *Sinanodonta amurensis* appeared less centered than that of either species of *Cristaria* (Fig. 2). The valve edge of the two species of *Cristaria* appeared to curve inward to the hinge more sharply than in *S. amurensis* (Fig. 2). Pits in the valves of the species of *Cristaria* tended to be smaller (0.5-1.5 µm diameter) than those in *S. amurensis* (0.8-2.0 µm) (Fig. 4).

The number and arrangement of microstylets on the hook differed in the one glochidium of each species examined. The two species of *Cristaria* had 15 to 20 large lanceolate microstylets that were more slender and shorter (maximum height 9.1-12.7 µm) and were arranged in 1-2 or 2-3 poorly-defined longitudinal rows (Fig. 3A,C). The glochidia of *Sinanodonta amurensis* had 10 or fewer large microstylets

Table 1. ANOVA of glochidial characters from *Cristaria herculea, Cristaria tuberculata, Sinanodonta amurensis,* and *Sinanodonta likharevi.* Analyses of Hook and Hook/Height do not include *S. likharevi.* Results of post-hoc tests are indicated in Figure 5 by letters.

Source	df	F-Ratio	p
Length	3	40.356	0.000
Error	74		
Height	3	17.210	0.000
Error	90		
Height/Length	3	36.452	0.000
Error	72		
Hinge	3	24.185	0.000
Error	79		
Hinge/Length	3	17.941	0.000
Error	71		
Hook	2	18.923	0.000
Error	38		
Hook/Height	2	6.863	0.004
Error	27		

that were more stout and longer (maximum height 13.6 μm) and were arranged in two discrete rows (Fig. 3E). The small microstylets in *S. amurensis* extended beyond the base of the hook to the outer margin farther than did the small microstylets in the two species of *Cristaria* (Fig. 3).

DISCUSSION

The question of whether *Cristaria herculea* and *Cristaria tuberculata* are different species has persisted among Russian malacologists (Kodolova and Logvinenko 1987, Prozorova and Sayenko 2001). Our examination of their glochidia suggests that these two forms differ significantly in glochidial hook and hinge characters, and we conclude that they are distinct species.

Our use of multiple glochidia from a single female of each species raised the question about variability of glochidia within a single female, among females of the same species, and among females of different species. If the variation of glochidia from different females of a single species were greater than the variation among females of different species, then glochidial features would not be reliable for discriminating among species. Our analysis of Hoggarth's (1999) data (reported above in the Methods section) suggests that for 10 species in the Anodontinae, characters of glochidia from a single female per species could be used for discriminating among species. We extrapolate that result on North American Anodontinae to Anodontinae from eastern Asia to conclude that significant differences in glochidia from different females indicate that the females are different species.

The glochidia of *Cristaria plicata* measured by Wu *et al.* (2000) had a smaller length (263.7 μm) and greater height (310.1 μm) resulting in more elongate valves (1.18 height/length ratio) than did those of *Cristaria herculea* and *Cristaria tuberculata* in this study. The hinge length in *C. plicata* (199.8 μm) was similar to what we found in *C. herculea,* and the number and arrangement of microstylets on the hook (15-18 microstylets arranged in 2 rows) was similar to what we found for *C. herculea* and *C. tuberculata.*

Our findings along with those of Wu *et al.* (2000) lead us to conclude that *Cristaria herculea, Cristaria tuberculata,* and *Cristaria plicata* are three separate species. This conclusion is in contrast to the conclusions of Haas (1969) and Brandt (1974), who synonymized *C. herculea* and *C. tuberculata* with *C. plicata.* Because Haas (1969) gave no justification for the synonymy, we cannot evaluate his evidence. On the other hand, it is not clear what species Brandt (1974) had. Brandt (1974: 279) wrote, referring to *C. plicata,* "As this species has semi-oval glochidia without hooks it cannot be placed among Anodontinae as Haas (1969), Vokes (1967: 213) a. o. suggested. ... There is only one true Anodontinae known from SE-Asia, *Sinanodonta woodiana* (Lea), on whose various forms innumerable 'species' are based." The species of *Cristaria* we studied, and as studied by Wu *et al.* (2000), certainly do have anodontine glochidia. What Brandt (1974) was calling *C. plicata* was probably not *C. plicata,* and therefore the synonymy is suspect.

Antonova and Starobogatov (1988) compared glochidia of *Cristaria tuberculata* from Primorye with the data on *Cristaria herculea* from Primorye made by Shadin (1938) and with the description of the glochidia of *Cristaria* spp. from Iturup Island (southern Kuril Islands) made by Inaba (1941, 1964). However, recent examination of material collected during the 1994-1999 expeditions of the International Kuril Island Project, a biotic survey and inventory of the Kuril Archipelago (Pietsch *et al.* 2001, 2003), indicated that the specimens from Iturup Island are actually members of the genus *Kunashiria* Starobogatov in Zatravkin, 1983 (Sayenko and Bogatov 1998, Sayenko 1999a, Bogatov *et al.* 1999, Sayenko and Ohara 2001). Consequently, some of the data on the genus *Cristaria* reported by Antonova and Starobogatov (1988) should be attributed to *Kunashiria.* This mistaken identity likely explains why Antonova and Starobogatov (1988) incorrectly concluded that glochidia of *C. tuberculata* have large microstylets arranged in one row at the end of the hook, whereas those of *C. herculea* have large microstylets in two rows. Even if they had identified the species correctly, the number of microstylet rows would not be reliable for species identification because the arrangement of microstylets on hooks in species of *Cristaria* is variable (arrangement in 1-2 to 2-3 poorly defined rows, this paper).

Comparison of our findings on *Cristaria herculea* and *Cristaria tuberculata* with findings on glochidia of two other species of *Cristaria* from Japan examined by Inaba (1941, 1964), *Cristaria discoidea* (Lea, 1834) and *Cristaria spatiosa* (Clessin, 1875), reveals the variability in height, width, shape, and hinge length of glochidia in this genus. The hinge lengths of the glochidia of *C. discoidea* and *C. spatiosa* (203 µm and 213-214 µm, respectively) and the heights of glochidia of *C. discoidea* (277 µm) are within the ranges of those of *C. herculea* and *C. tuberculata.* However, the length of glochidia of *C. discoidea* (259 µm) is less than those of *C. herculea* and *C. tuberculata* and the heights and lengths of glochidia of *C. spatiosa* (312-330 µm and 284-307 µm, respectively) are considerably greater than those of the Russian *Cristaria* we studied (271-300 µm height, 270-286 µm length). Furthermore, glochidia of *C. discoidea* and *C. spatiosa* are slightly more elongate (height/length = 1.07 and 1.04-1.12, respectively) than those of *C. herculea* and *C. tuberculata* (1.01-1.06).

In addition to the species of *Sinanodonta* examined here, two other species of *Sinanodonta* have been studied extensively: *Sinanodonta calipygos* (as *Anodonta woodiana calipygos;* Inaba 1941, 1964), and *Sinanodonta woodiana* (as *A. woodiana lauta; A. woodiana lauta tumens;* and *A. woodiana;* Inaba 1941, 1964, Kwon *et al.* 1993, Park and Kwon 1993). In Japanese specimens, the heights of the glochidia of *S. calipygos* (298-313 µm) and *S. woodiana* (277-303 µm) and the lengths of the glochidia of *S. woodiana* (243-268 µm) are larger than those of the Russian *Sinanodonta amurensis* and *Sinanodonta likharevi* we studied. On the other hand, the lengths of the hinges of the glochidia of Japanese *S. calipygos* (180-191 µm) are in the same range as those of *S. likharevi* but considerably smaller than those of *S. amurensis.* Glochidia of *S. woodiana* from Korea do not differ from the Russian individuals of *Sinanodonta* in any measurements. Although the size of the glochidia of all species of *Sinanodonta* is mostly in the same range, the island specimens have larger glochidia than the continental specimens. Moreover, glochidia of the species of *Sinanodonta* studied in Japan by Inaba (1941, 1964) are more elongate (height/length = 1.18-1.23 for *S. calipygos,* and 1.13-1.14 for *S. woodiana*) than the species of *Sinanodonta* studied in Korea (1.04 for *S. woodiana* [Kwon *et al.* 1993, Park and Kwon 1993]) and in Russia (this paper). We conclude that the glochidia of *S. amurensis* are the smallest and that the glochidia of the Japanese *S. woodiana* are the largest of the species of *Sinanodonta* studied.

Regarding the hook features, the large microstylets of glochidia of *Sinanodonta woodiana* from Korea were centrally arranged in two uneven rows (Park and Kwon 1993), as we found in the glochidia of *Sinanodonta amurensis* from eastern Russia.

In contrast to other species of Anodontini (*Anemina*-group species), in which the height of the glochidia is less than the length and the glochidia are weakly asymmetric (Jeong 1989, Lee *et al.* 1989, Jeong *et al.* 1993, Kwon *et al.* 1993, Park and Kwon 1993, Chernyshev 1998, Sayenko 1999b), the glochidia of *Sinanodonta amurensis* and *Sinanodonta likharevi* were elongate (height > length), as were those in the species of *Cristaria* we examined. Furthermore, in the glochidia of *S. amurensis,* the pits were regularly distributed over the entire external and internal surfaces. This pit distribution is in contrast to that in glochidia of other species of Anodontini. For example, in the *Anemina*-group of species, the internal valve surface where the adductor muscle attaches either lacks pits, has fewer pits, or the pits are smaller than the pits on other parts of the glochidial valves (Chernyshev 1998).

In summary, we found significant differences in characters of multiple glochidia from a single parent of each species. Our analysis of data from Hoggarth (1999) showed significant variation in glochidial features among species, but no significant variation in glochidia among parents of the same species, suggesting that significant glochidial differences across parents would indicate different species. Consequently, our finding significant differences in multiple glochidia from single parents of *Cristaria herculea* and *Cristaria tuberculata* and differences in multiple glochidia from single parents of *Sinanodonta amurensis* and *Sinanodonta likharevi* support the recognition of these taxa as distinct species. Glochidial features of *Sinanodonta* spp. differ from those of other members of the tribe Anodontini (including the genus *Anemina*), casting doubt on whether *Sinanodonta* belongs in the tribe Anodontini. Glochidial similarities between *Sinanodonta* spp. and *Cristaria* spp. hint that *Sinanodonta* might belong in the tribe Limnoscaphini along with *Cristaria.* Future phylogenetic analyses that include glochidial characters of more members of the subfamily Anodontinae Ortmann, 1910 will provide additional insight into the relationships among the taxa.

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Structure and composition of subepidermal granules from *Aplysia californica* Cooper, 1863 (Gastropoda: Opisthobranchia)

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Abstract: Subepidermal granules from *Aplysia californica* were studied to learn their structure, chemical composition, and possible function. Granules were examined in tissue sections using light microscopy, and isolated granules were examined using scanning electron microscopy. Granules were located 100-200 µm beneath the surface and were present at concentrations of over $200/\text{mm}^2$ of epidermis. The largest granules were about 0.25 mm in their longest axes. Granules appeared solid in construction and seem composed primarily of calcium carbonate. The contribution of these granules to body coloration is discussed.

Key words: *Aplysia,* Granule, Biomineralization, Shell, X-ray analysis

Biomineralization has been studied in many invertebrates, including members of the phylum Mollusca where much research has concerned shell formation, demineralization, and repair (Watabe *et al.* 1976, Carter 1990). Among opisthobranch molluscs, two morphological types of "spicules" have been described from various tissues in nudibranchs (Cattaneo-Vietti *et al.* 1995). Mineralized granules were noticed beneath the epidermis of *Aplysia californica* Cooper, 1863 during other studies of this animal. Although Eales (1960) briefly mentioned such granules in this genus, she provided no details, and no illustrations appear to have been published elsewhere. Therefore we undertook to isolate and describe the granules from *A. californica.*

METHODS

Adult individuals of *Aplysia californica* (>200 g) were obtained from Alacrity Marine Biological (Redondo Beach, CA). Samples of epidermis were processed in two ways to obtain photomicrographs of the granules *in situ.* Several fresh samples were excised from the parapodial edge, the pigmented epithelial cell layer was carefully peeled away, and the remaining tissue was exposed to a solution of 25% bleach in buffered sea water for 60 min to digest some of the cells overlying the granules. Two fresh samples of the tips of the rhinophore were fixed in 10% buffered formalin, dehydrated with ethanol, and cleared with xylene. Additional samples of rhinophores were fixed in buffered formalin, dehydrated and cleared as above, and embedded in paraffin. These samples were then sectioned at 10 µm and stained with hematoxylin and eosin to enable histological examination of granules and surrounding subepidermal tissue using light microscopy (LM).

To isolate granules for further study, fresh samples of parapodial tissue containing granules were placed in a test tube containing 1 ml of 25% bleach solution and agitated periodically for 60 min. This duration was determined to be adequate to isolate the granules by a time-study experiment. The partially digested tissue was removed, and the remaining granules were allowed to settle. The solution was decanted and the granules were rinsed twice with distilled water, followed by three rinses in 75% EtOH. Samples of granules were transferred to scanning electron microscope (SEM) stubs and allowed to air dry before sputter coating with gold. Images were obtained at 5 keV with an Aspex PSEM-2000. The minimum axis length, maximum axis length, and area of the granules were measured in planar view from SEM photomicrographs using NIH Image (v. 1.62). X-ray elemental analyses were done on granules (over 50 samples) from the parapodium of *Aplysia californica* and on its vestigial shell (five samples) for comparison, using the SEM at 20 keV with an acquisition time of 90 sec.

RESULTS

Subepidermal granules were observed in *Aplysia californica* in the rhinophore, oral tentacles, siphon tip, posterior foot, and parapodia, and were typically found in clusters. They appeared white and were visible to the naked eye. No differences in granule size or other features were apparent across body areas, but differences in abundance were clearly present and contributed to the body's overall color pattern. Although juveniles were not studied here, one of us (PVH) has observed the same pattern of white granules in both juvenile and adult *Aplysia brasiliana* Rang, 1828.

Granules were clearly visible within samples of both parapodial and rhinophoral tissue (Figs. 1A and B, respectively). The granules in Figure 1A had an estimated concentration of 230 granules/mm^2 of skin surface. The granules were located 100-200 µm beneath the epidermis, embedded in connective tissue (Fig. 1C). They were strongly basophilic, and some appeared to have one or more distinct oval areas within them that took up little stain, giving the appearance of internal chambers (Fig. 1D). Evidence of a latticework could be seen in some sections with LM at 1000×. The surfaces of granules appeared rough when viewed with SEM (Fig. 2E). The granules were solid when viewed using the SEM, with no apparent variation in structure (Fig. 2F).

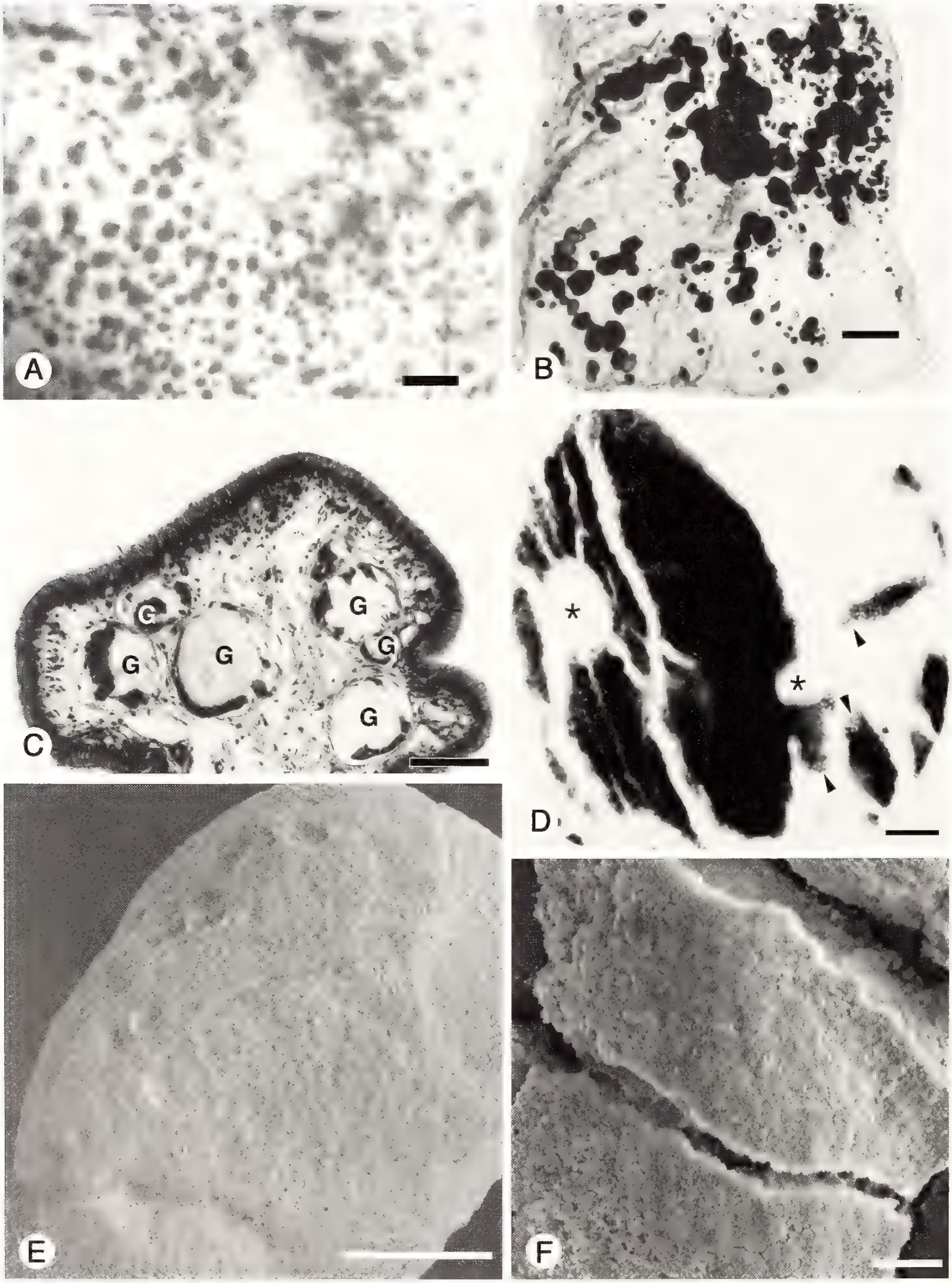

Figure 1. Subepidermal granules from *Aplysia californica*. (A) Light microscopy (LM) surface view of partially digested parapodial tissue showing granules at different depths. Scale bar = 200 µm. (B) LM surface view of rhinophore tissue that was fixed and cleared with xylene. Scale bar = 200 µm. (C) LM of granules in section of tissue from edge of rhinophore tip. The six largest granules are labeled G; additional smaller granules are present. Some material was lost from all granules during sectioning. Scale bar = 100 µm. (D) LM of single granule in tissue section, showing multiple fractures, two apparent chambers (asterisks), and traces of latticework (arrows). Scale bar = 10 µm. (E) SEM of a granule revealing its rough surface. Scale bar = 20 µm. (F) Higher magnification SEM of a fractured granule showing solid internal structure. Scale bar = 10 µm.

Individual granules varied in shape and size. The average dimensions (minimum × maximum axis) of a sample of 257 granules were 45 × 155 μm. The smallest granule measured 3.5 × 3.5 μm and the largest granule was 131 × 237 μm. The average area of the granules (planar surface) was 1,536 μm^2, with a range from 10 to 24,265 μm^2 (Fig. 2).

The x-ray elemental analysis of granules revealed the presence of calcium, carbon, chlorine, magnesium, oxygen, and sodium (Fig. 3 top). The only common elements between the granules and the vestigial shell (Fig. 3 bottom) were calcium, carbon, magnesium, and oxygen, but the signature peaks for these elements varied greatly between the two structures. The vestigial shell of *Aplysia californica* is not heavily mineralized, which may account for the short signature peak for calcium. X-ray analyses of the vestigial shell also indicated the presence of bromine. Minute traces of sulfur were also detected, which could be due to sulfide bonds within the protein matrix of the paper-thin shell.

DISCUSSION

The sizes of the granules from *Aplysia californica* we measured (3.5-237 μm) were similar to the size range (0.5-200 μm) reported for granules in other molluscs (Watabe *et al.* 1976). Furthermore, x-ray analysis of the surface chemistry of granules from *A. californica* indicated that they were similar to the composition of granules in other molluscs (Burton 1972, Roinel *et al.* 1973, Watabe *et al.* 1976, Simkiss 1980, Silverman *et al.* 1991). The occurrence of granules in distinct clusters, such as found in *Aplysia*, was also reported in the bivalve *Hyridella depressa* (Lamarck, 1819) (Byrne

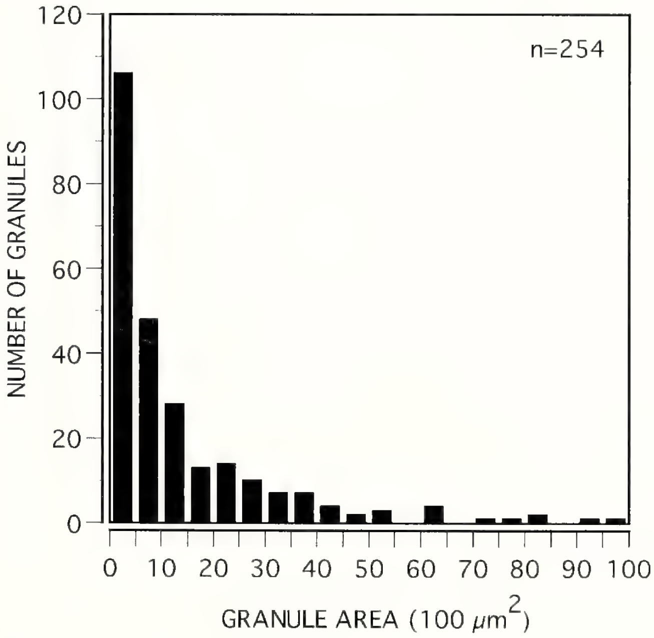

Figure 2. Size-frequency distribution of granule sample from *Aplysia californica*. Three granules with planar surface areas greater than 10,000 μm^2 were omitted from this graph.

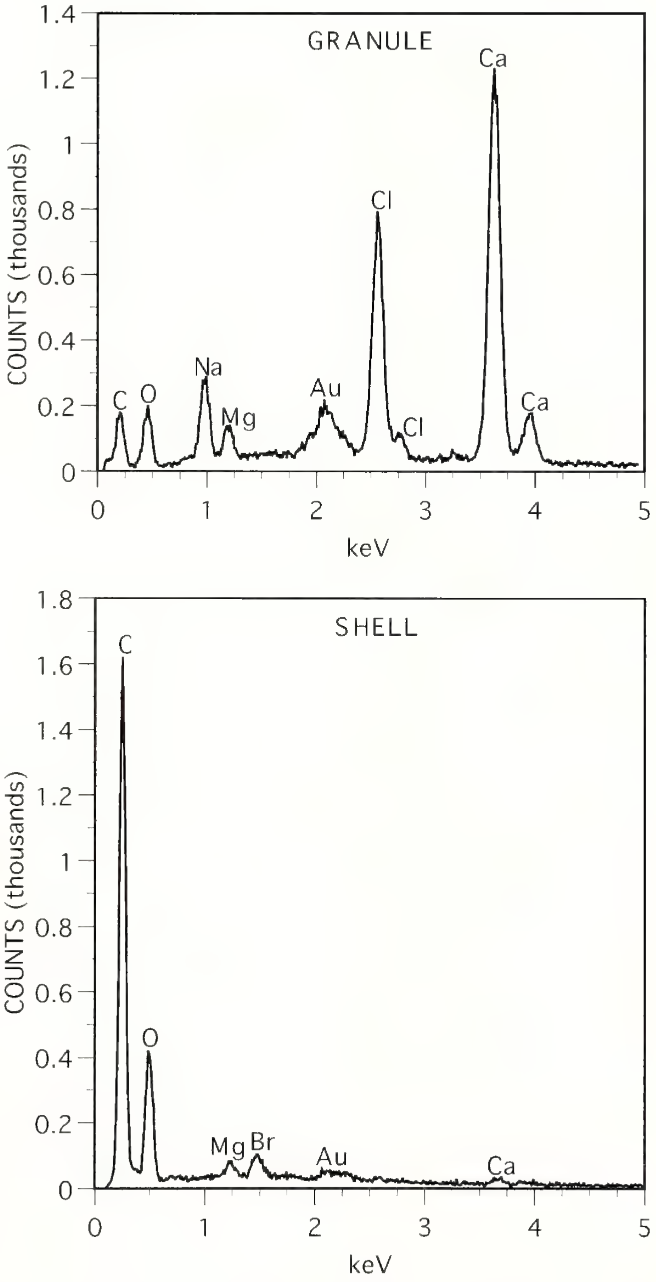

Figure 3. SEM x-ray elemental analysis of the two biomineralized structures in *Aplysia californica*, the granules (top) and vestigial shell (bottom). Au, gold; Br, bromine; C, carbon; Ca, calcium; Cl, chlorine; Mg, magnesium; Na, sodium; O, oxygen

2000). The appearance of internal chambers in granules after conventional histological processing (Fig. 1D) contrasted with the solid appearance of granules viewed with SEM (Fig. 1F). The internal chambers could be dissolution artifacts. Byrne (2000) attributed some granule structural changes to tissue processing steps.

Despite the above similarities, several of the functions proposed for the mineralized granules of other molluscs do not seem applicable to *Aplysia californica*. Watabe *et al.* (1976) and Carter (1990) suggested that granules provide a store of minerals for shell formation and repair. However, this function seems illogical for *A. californica* as it has an internal vestigial shell and calcium is rarely limiting in sea water. Another proposed function of molluscan granules has been the supply of calcium for egg capsules (Watabe *et al.* 1976, Silverman *et al.* 1991). This also seems unlikely for *A. californica* because its egg capsule is gelatinous and probably contains little calcium. Burton (1983) suggested that the granules may buffer against acidosis when an animal is in anaerobic conditions; *A. californica* is typically found in well-aerated water, however animals sometimes survive intertidal exposure, especially if lodged in crevices or covered with vegetation. Cattaneo-Vietti *et al.* (1995) noted that the calcareous spherules from a nudibranch's skin could be related to variations in ion concentration caused by pH variations due to dermal gland activity. Byrne (2000) suggested that the granules of a freshwater bivalve may be a byproduct of ionic regulation or (other) biomineralization processes.

The ability of *Aplysia californica* to produce and retain a biomineralized structure may have an adaptive function totally different from those suggested above. The granules of *A. californica* are white, occur in clusters, and are located very close to the body surface, all of which make them clearly visible externally. Thus, their contribution to the animal's appearance must be considered. This herbivorous animal lives on the bottom amid benthic vegetation, where similar light-colored lines, reticulations, and small patches are found on seagrass, drift algae, and other bottom materials. Of the ions present in sea water that can form a white pigment or mineralized structure (antimony, barium, calcium, lead, magnesium, titanium, and zinc), calcium is abundant and relatively easy to precipitate in the form of calcium carbonate. Indeed, elemental analysis of the granules (Fig. 3) indicated that calcium carbonate was probably a major component. The other elements listed above are either present in sea water at much lower concentrations or precipitate less readily. Thus, we suggest that the granules make an ecologically significant contribution to the animal's appearance, possibly related to camouflage.

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The freshwater mussels (Mollusca: Bivalvia: Unionidea) of northern Nebraska: The Missouri, Niobrara, and White River basins

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Abstract: A survey of 46 sites in northern Nebraska documented six unionids. Species diversity was greatest in the Missouri River in northeastern Nebraska; the diversity of the waters of interior northern Nebraska was extremely low, with only three species occurring in the vast region extending from 10 km west of the Missouri River to the Wyoming border. Currents in the interior are generally strong and substrates often consist of shifting sand. Bivalves were absent from such environments. Mussels were restricted to habitats with stable substrates and slow to moderate current, including slow headwater portions of the major interior rivers, some creeks, reservoirs and ponds, and backwaters and side channels along the major rivers. In interior northern Nebraska, the value of many of these environments for unionid molluscs is declining due to grazing and water removal.

Key words: unionids, species diversity, bivalves, environmental conditions.

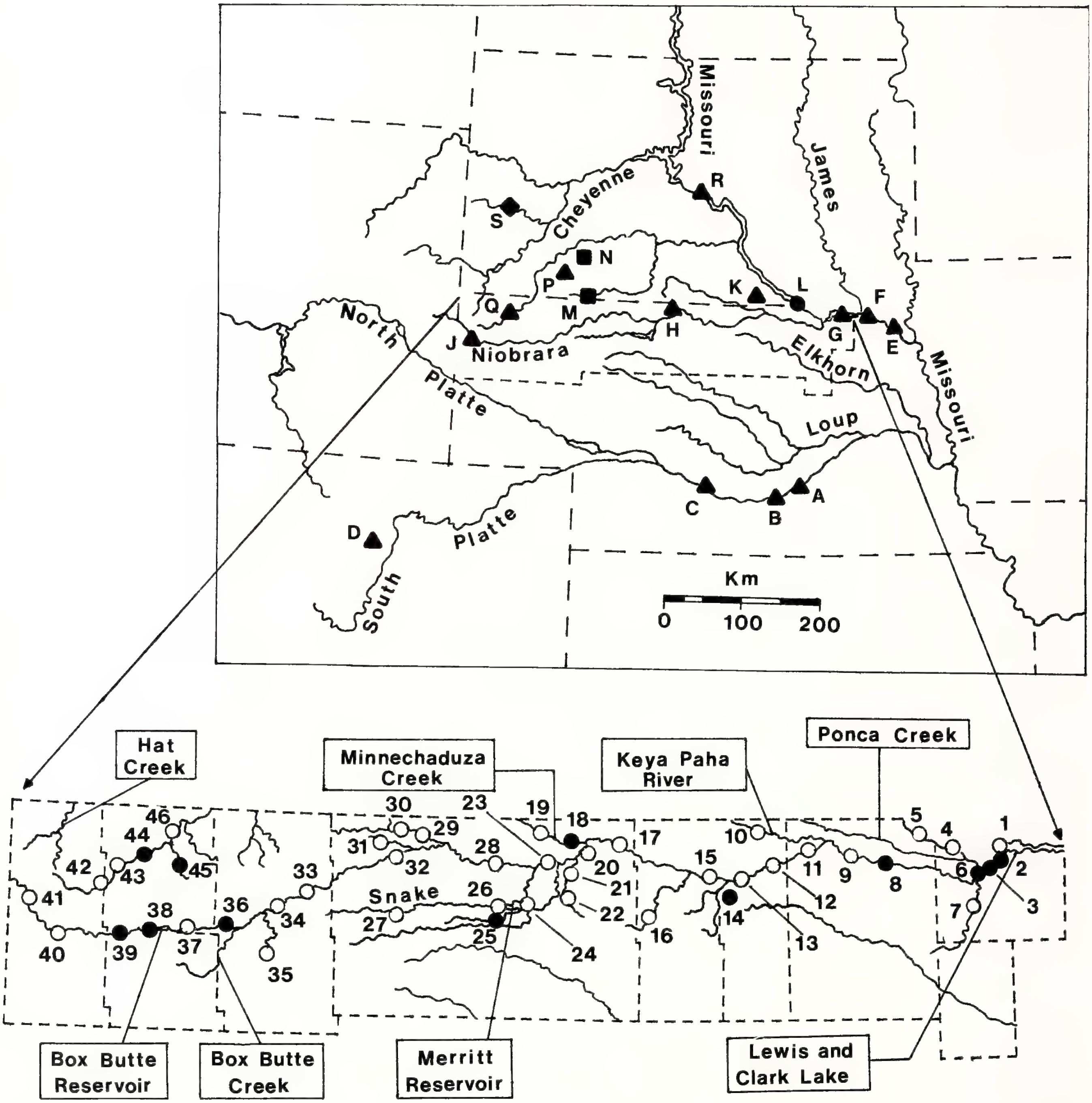
The unionid molluscs of northern Nebraska are poorly known, and no previous general survey of the region exists. The only nineteenth century paper on the unionids of the region is that of Hayden (1862), who collected one species from the Nebraska portion of the White River. The rivers of this area are not mentioned by Aughey (1877) in his paper on the molluscs of Nebraska, and the only recent literature on mussels in the interior of the region is limited to a single paper on the Niobrara Basin (Freeman and Perkins 1997).

This study is a portion of a larger project initiated in 1972 to document the freshwater mussels of Nebraska and to gain an understanding of their distributions. The purpose of this paper is to present results of the northern Nebraska portion of this project and to summarize all previous work in the region. The primary emphasis of this survey was to obtain baseline data for the region through collection of specimens over as wide a geographic area and in as great a range of potential habitats as possible.

In this paper, northern Nebraska is defined as the Niobrara Basin and all regions northward as well as a small reach of the Missouri River southeast of the mouth of the Niobrara and just above the upper end of Lewis and Clark Lake (Fig. 1). The region is located entirely within the Great Plains physiogeographic province of North America (Fenneman 1931). Topography is generally rolling, with much greater relief in the northwestern corner of the state. The Missouri River and the eastern half of the Niobrara River flow through relatively deep, generally narrow valleys. Native vegetation consists primarily of short grasses, with forested areas restricted to portions of the major valleys and the Pine Ridge area of northwestern Nebraska. The region is sparsely populated and the economy is based upon ranching and agriculture.

All the rivers and streams in the region eventually drain into the Missouri River. Beginning several kilometers east of the mouth of the Niobrara and extending eastward for 68 km, the Missouri River has been impounded to form Lewis and Clark Lake. The 72 km reach extending from above this reservoir west to the base of the Fort Randall Dam in South Dakota is one of only two reaches of the river bordering Nebraska that have not been channelized or impounded. A 60 km section of this reach has been designated as a National Scenic River. The unchannelized Missouri River contains side channels, islands, and backwater areas, features largely or entirely eliminated from channelized reaches of the river to the south and east. In the study area, most of these features are found along the South Dakota banks of the river; however, side channels and backwaters are present along the Nebraska shore below the mouth of the Niobrara River at the western end of Lewis and Clark Lake.

The largest river in the interior is the Niobrara River, with a total drainage basin in Nebraska of 30,108 km^2. This river extends from eastern Wyoming across northern Nebraska and empties into the Missouri River in northeastern Nebraska. The Nebraska portion of the basin includes approximately 784 km of the Niobrara River, as well as all of the Snake River and the lower reaches of the Keya Paha River. The Niobrara is very narrow and slow flowing in its headwater reaches, but broadens and increases in velocity to the east. The eastern half of the river is often a braided stream with a considerable current. Except within holes, the Niobrara is rarely more than 1.5 m in depth. The extreme northwestern corner of the state includes the headwaters of the White River and Hat Creek and other small tributaries of the Cheyenne River with drainage basins of 4,287 km^2 and 1,159 km^2, respectively. The final major component to the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Loup
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Platte
South
Km
0
100
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Hat Creek
Minnechaduza Creek
Keya Paha River
Ponca Creek
Snake
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46
Box Butte Reservoir
Box Butte Creek
Merritt Reservoir
Lewis and Clark Lake

Figure 1. Above: The Northern Nebraska region with locales of collection sites referenced in the text. Filled circles indicates sites collected by the author; filled triangles reference published sources; filled squares indicate unpublished museum records; and the filled diamond references an anecdotal report. Sources for published sites and additional site information: A, Roedel (1990); B, Freeman and Perkins (1992); C, Peyton and Maher (1995); D, Ellis and Keim (1918); E, Clarke (1996), Perkins (2000); F, Hoke (1983), sites 3-5; G, Hoke (1983) sites 1-2; H and J. Freeman and Perkins (1997); K, Ponca Creek (Backlund 2000); L, Collection by author below Fort Randall Dam; M, Little White River (specimens at University of Colorado Museum of Natural History); N, Yellow Medicine Creek (Over 1915); P, Redwater Creek (specimens at the Philadelphia Academy of Sciences and the National Museum of Natural History); Q, Hayden (1862); R, Lake Sharpe (Backlund 2000); S, Rapid Creek, anecdotal locality. Below: Survey sites collected in northern Nebraska. Filled circles indicate productive sites, open circles indicate non-productive sites.

region's drainage system is a 1,005 km^2 area in eastern Nebraska that includes the Missouri River floodplain and some small direct tributaries of the Missouri River. The largest of these is Ponca Creek with a Nebraska basin of 981 km^2. This stream originates in South Dakota and flows through northeastern Nebraska to its confluence with the Missouri River a few kilometers above the mouth of the Niobrara.

METHODS

The survey relied heavily upon input from local conservation officers and residents to locate unionid populations. In 1976 conservation officers in Nebraska and South Dakota were contacted via a questionnaire to obtain the locations of unionid populations. Responses provided a number of potential collection localities in northern Nebraska and adjacent South Dakota. In 1998 landowners at most access points in the western portion of the study area were contacted again via a questionnaire to locate additional populations, as well as to obtain permission to collect. Area residents were also interviewed during the course of the survey.

Fieldwork was conducted in northern Nebraska in 1980, 1998, and 2000. Collection sites were determined primarily by physical access conditions and the ability to obtain consent for collection. The infrequency of primary roads and the poor condition of secondary roads greatly limited physical access to rivers and streams. Landowner opposition to and even hostility toward environmental research further severely constricted survey activities. Therefore, it was not possible to collect in many streams that might have provided favorable habitats. Despite these limitations, an attempt was made to collect throughout the region and to sample each of the various aquatic environments present. Habitats sampled included backwaters, side channels, headwater reaches, and lower and middle reaches of large rivers; creeks; and ponds, lakes, and reservoirs.

Sites were sampled until either diversity plateaued or until the accessible collection area had been surveyed. Time spent at each locale varied from one half to three hours, and averaged about one hour. Sampling was qualitative, not quantitative, and each site was sampled by the author on one occasion only. Collections were made by hand or with a garden rake, usually under low water conditions. To conserve limited unionid resources, fresh shells were collected in preference to live individuals. Most live specimens were identified and immediately returned to their substrates. Specimens were first identified by the author and verified as needed by Dr. David H. Stansbery of the Museum of Biological Diversity, Ohio State University, U.S.A. All specimens collected in this study have been or will be deposited in the collections at the Museum of Biological Diversity in Columbus, Ohio, U.S.A. The taxonomy utilized in this paper follows Turgeon *et al.* (1998). A number of museums around the country were also visited and their collections examined to locate specimens from the study area. These institutions are acknowledged in a later section of this paper.

RESULTS

Collection activities were conducted at 46 sites (Fig. 1), 5 along the Missouri River, 36 in the Niobrara Basin and 5 in the White Basin. Due to an inability to obtain landowner consent for collection activities, no sites were visited within the Nebraska reaches of the Cheyenne Basin (Hat Creek) or along Ponca Creek. Unionids were uncommon in northern Nebraska and occurred at only twelve sites, or 26.1% of the total sites. Eleven sites yielded identifiable unionids; bivalves were represented at one site by a single fragment of an unidentifiable thin-shelled species. Mussels were also reported by local residents at four additional locales, although collecting efforts were unsuccessful at those sites. Forty-four of the collection locales were sampled solely by the author. Site 45 was first visited in 1973 by a Nebraska Game and Parks Commission official, who donated his specimen to the author. The author visited this site in 1980 with the same results. Specimens from site 14, a farm pond along Short Pine Creek, were collected by a local resident and given to the author.

Species richness was very low, with an average of 0.46 species per site and only 1.75 species per productive site. Only three sites produced more than one species. Two of these sites were along the Missouri River and the third was a backwater area along the Niobrara River only a few miles from its confluence with the Missouri. Site 2 was the most productive locale, with five species collected. All species were collected live or as recent shells, suggesting that the limited richness of the area was generally stable. This contrasts with the condition of the unionid fauna in eastern Nebraska, where declines of 48 to 61 percent have been reported in some basins (Hoke 1994b, 1996).

Six unionids were collected in this study (Table 1). Five species were found in the Missouri River and all represent new records for this reach. Two species, *Potamilus ohiensis* (Rafinesque, 1820) and *Quadrula quadrula*(Rafinesque, 1820), were entirely restricted to the Missouri River. Four species were collected from the Niobrara Basin and three; (*Anodontoides ferussacianus* [Lea, 1834], *Lasmigona complanata complanata* [Barnes, 1823], and *Leptodea fragilis* [Rafinesque, 1820]) are first reported in this study. The fourth, *Pyganodon grandis* (Say, 1829), was collected by Freeman and Perkins (1997) from one site in the Niobrara Basin. Two species, *A. ferussacianus* and *P. grandis,* were recovered from the White River Basin. The latter species is first reported from the White Basin in this study, while the collection of the former species constitutes the first record for the

Table 1. Unionid molluscs collected from Northern Nebraska listed by collection site: F = fresh shell; L = live; R = recent shell; S = subfossil or chalky shell; WD = weathered shell.

		White Basin			Niobrara Basin									Missouri River			
	Site number	45	44		6	8	14	18	25	36	38	39		2	3		
	Year(s) collected	1973* & 1980	1998		1998	1980	1980*	1980	1998	1998	1980	1998		2000	2000		
Species Collected	Habitat[1]	res	hwtr	Total	bwtr	ox	pnd	res	ck	hwtr	hwtr	hwtr	Total	bwtr	sc	Total	Total study
Anodontoides ferussacianus (Lea, 1834)		–	R	1	–	–	–	–	–	WD	L	L	3	–	–	–	4
Lasmigona c. complanata (Barnes, 1823)		–	–	–	F	–	–	–	–	–	–	–	1	R	R	2	3
Leptodea fragilis (Rafinesque, 1820)		–	–	–	F	–	–	–	–	–	–	–	1	R	R	2	3
Potamilus ohiensis (Rafinesque, 1820)		–	–	–	–	–	–	–	–	–	–	–	–	R	R	2	2
Pyganodon grandis (Say, 1829)		L	–	1	F	L	F	L	–	–	–	–	4	R	R	2	7
Quadrula quadrula (Rafinesque, 1820)		–	–	–	–	–	–	–	–	–	–	–	–	R	–	1	1
Unidentifiable fragment		–	–	–	–	–	–	–	S	–	–	–	1	–	–	–	1
Total species		1	1	2	3	1	1	1	1	1	1	1	10	5	4	9	21

[1] Habitats: bwtr = backwater; ck = creek; hwtr = headwater; ox = oxbow; pnd = pond; res = reservoir; riv = river; sc = side channel.

* Not collected by author.

Nebraska portion of the basin in over 140 years. The species recovered from the region are discussed in detail below.

The most frequently encountered bivalve was *Pyganodon grandis.* This unionid was often collected from manmade habitats such as reservoirs and ponds, but was also recovered from backwater areas along the Missouri and Niobrara rivers, a side channel of the Missouri River, and an oxbow lake along the Niobrara River. The mussel inhabited substrates composed of mud, stable sand, or mud and sand, generally in little or no current, in water ranging from several centimeters to one meter in depth. It was almost certainly present at greater depths in some reservoirs. Due to its frequent association with manmade or artificial habitats, this species is probably now more common in the study area than prior to the region's agricultural development. The presence of this unionid in a small isolated reservoir in the upper White River Basin in northwestern Nebraska may be the result of a recent introduction. Local residents also reported large mussels, probably of this species, from Merritt Reservoir, an impoundment of the Snake River, and from a number of smaller lakes and reservoirs in the Niobrara Basin.

Anodontoides ferussacianus was the second most-widely distributed mussel. It was the only bivalve found in the headwaters of the Niobrara and White rivers, and probably occurs in some of the tributary creeks in the region as well. *Anodontoides ferussacianus* was collected from streams with slow to moderate current, usually in mud substrates, at depths of 15 to 50 cm. This mussel was the only species recovered from the interior that was not also collected in the Missouri River.

Lasmigona c. complanata and *Leptodea fragilis* were both recovered from the same three sites. Two sites were along the Missouri River, a backwater and a side channel, and the third was a sheltered backwater along the Niobrara River, several miles above its mouth. In the Niobrara, similar habitats extend upstream for 15 to 20 km above site 6 and it is likely that both species range at least this far to the west. The bivalves were found in mud, sand and mud, and sand substrates at depths ranging from a few centimeters to one meter in quiet to moderately flowing current. *Lasmigona c. complanata* was always relatively uncommon. *L. fragilis,* rare in the Niobrara, was extremely abundant at some sites along the Missouri River.

Potamilus ohiensis was restricted to two locales along the Missouri River, but was quite numerous when present. The mussel was collected from mud, as well as mud and sand substrates in a large backwater area, where currents were very slow. It was also found deeply embedded in the sandy substrates of a shallow side channel of the Missouri River. Although this site was mostly dry at the time of collection, normal currents were probably somewhat faster than the backwater area. Further collection efforts along the lower Niobrara may reveal this species in backwater areas of that stream as well.

A single set of paired valves of *Quadrula quadrula* was collected from a backwater area along the Missouri River. This locale presently constitutes the northwestern-most extent of the species' distribution in the Missouri River.

The only museum records from the immediate study area are located at the University of Nebraska State Museum in Lincoln, Nebraska, and pertain to Freeman and Perkins (1997) Niobrara River paper. There are also a number of museum specimens from the region immediately north of the study area. These records as well as those of other authors are discussed below. The related collection locales are shown in Figure 1.

DISCUSSION

Literature and museum review

The sole publication on the mussels of the White River in northern Nebraska is that of Hayden (1862) who reported *Anodontoides ferussacianus* (as "*U. ferussaciana*, Lea"), and deposited a specimen in the U. S. National Museum (No. 86528). There are also a number of records from the White River Basin in South Dakota. Over (1915) reported *A. ferussacianus* from Yellow Medicine Creek (now Medicine Root Creek) in Washabaugh County (now Shannon County), South Dakota, and unpublished vouchers collected by him from Redwater Creek in Washabaugh County (now Jackson County) are deposited at the Field Museum of Natural History (No. 44460) and at the Academy of Natural Sciences (No. 111099). A further record from the Little White River in Bennett County, South Dakota (No. 29888), is at the University of Colorado Museum of Natural History.

Freeman and Perkins (1997) reported one population each of *Pyganodon grandis* and *Strophitus undulatus* (Say, 1817) from the Niobrara Basin. *Pyganodon grandis* was collected below a small reservoir on Minnechaduza Creek (a tributary of the Niobrara River), and *S. undulatus* from the headwaters of the Niobrara River. The current study found additional populations of the former species in the Niobrara Basin, but not of *S. undulatus*.

Strophitus undulatus has been confirmed for eastern Nebraska (Hoke 1994b, 1995, 1996) but its presence in western portions of the central plains has been subject to question. The species has been reported for St. Vrain Creek, a tributary of the South Platte River near Longmont, Colorado (Ellis and Keim 1918), and for the Platte River in central and west central Nebraska (Roedel 1990, Freeman and Perkins 1992). No extant vouchers support the former study, and the identification has been questioned by Watters (1994). Freeman and Perkins (1992) were unable to locate any identifiable voucher from Roedel's study, and although they also reported the species for the Platte River based upon their collection of a single "half-valve," were tentative of their identification. The first confirmed report for the region is that of Peyton and Maher (1995) for the Platte River and adjacent connecting canals west of Overton, Nebraska, based upon a genetic analysis by Dr. Hsiu-Ping Liu, Brian Kreiser and Dr. Shi-Kuei Wu of the University of Colorado. Dr. G. Thomas Watters recently examined vouchers from the study of Freeman and Perkins (1997) and confirmed their identification of *S. undulatus*.

There are no previous records for the reach of the Missouri River within the study area, but reports document the fauna below and above that reach. The results of the current study are contrasted with those from adjacent reaches of the Missouri River in Table 2, and suggest a decrease in the diversity of unionid species from east to west. Fourteen species are known from the reach below Lewis and Clark Lake, but only six have been confirmed to the west. The decline in species diversity above the Gavin's Point Dam suggests that the dam may serve as a faunal barrier for a number of unionids.

An old chalky valve of *Lampsilis cardium* (Rafinesque, 1820) was recovered by the author in 1982 from the Missouri River about two miles south of the Fort Randall Dam in South Dakota. This is the first record of the species from the Missouri River, although the shell may have washed out of Randall Creek a few kilometers upstream. It is also the westernmost record for this mussel in South Dakota. Its presence in a region north of and contiguous to the study area suggests that *L. cardium* may be a component of the unionid fauna of northern Nebraska.

Backlund (2000) collected *Lasmigona* c. *complanata, Ligumia subrostrata* (Say, 1831), *Pyganodon grandis,* and *Uniomerus tetralasmus* (Say, 1831) from the upper portion of Ponca Creek in Gregory County, South Dakota. The lower (Nebraska) portion of the creek may also contain some of these species.

No mussels have been reported for the Cheyenne River Basin, almost certainly due to an absence of collection effort. In response to a 1976 questionnaire, one conservation officer reported mussels as common in Rapid Creek (a tributary of the Cheyenne River) at Rapid City, South Dakota.

Analysis of survey data

The habitats for unionid molluscs in northern Nebraska are far from optimal, and the general scarcity of unionids was not surprising. The distribution of bivalves was highly concentrated in geographically restricted habitat types (Table 3). Although 30 of the 46 sites in northern Nebraska

Table 2. Unionids reported from the Missouri River along and near the Nebraska - South Dakota border.

	West <------------------------------					------------------------------> East
	Lake Sharpe	Above Lewis & Clark Lake to Fort Randall Dam	Lewis & Clark Lake	Below Lewis & Clark Lake		
Taxa	Backlund (2000) Sites 1-6	Hoke Current study	Hoke (1983) Sites 1-2	Clarke (1996) 17 Sites	Hoke (1983) Sites 3-5	Perkins (2000) 47 Sites
Amblema p. plicata (Say, 1817)	–	–	–	–	–	*
Anodonta suborbiculata (Say, 1831)	–	–	–	–	–	L
Arcidens confragosus (Say, 1829)	–	–	–	–	–	D
Lampsilis cardium (Rafinesque, 1820)	–	*	–	–	–	–
Lampsilis siliquoidea (Barnes, 1823)	–	–	–	–	–	D
Lampsilis teres (Rafinesque, 1820)	–	–	–	–	–	D
Lasmigona c. complanata (Barnes, 1823)	L	F	R	F	L	L
Leptodea fragilis (Rafinesque, 1820)	L	F	F	L	L	L
Leptodea leptodon (Rafinesque, 1820)	–	–	–	–	F	–
Potamilus alatus (Say, 1817)	–	–	–	L	L	L
Potamilus ohiensis (Rafinesque, 1820)	L	F	F	–	L	L
Pyganodon grandis (Say, 1829)	L	F	L	L	L	L
Quadrula quadrula (Rafinesque, 1820)	–	R	R	–	R	L
Strophitus undulatus (Say, 1817)	–	–	–	–	–	*
Toxolasma parvus (Barnes, 1823)	–	–	–	–	–	*
Tritogonia verucosa (Rafinesque, 1820)	–	–	–	–	M	–
Truncilla donaciformis (Lea, 1828)	–	–	–	–	R	D
Truncilla truncata (Rafinesque, 1820)	–	–	L	–	L	L
Species confirmed for Missouri River	4	5	6	4	10	12

Condition of shells: D = dead; F = freshdead; L = live; M = museum specimen; R = recent.

* Shell found in Missouri River that may have washed into river from a tributary stream.

were in major rivers, only a handful produced unionids, and these were concentrated in three habitats, headwater reaches, side-channels, and backwaters. Forty percent of the headwater sites were productive; 19% of all species occurrences are represented at these sites. Three sites, a side-channel and two backwater areas, produced 57% of all species occurrences. In contrast, the 17 main channel river sites were devoid of unionids.

Table 3. Summary of study results from northern Nebraska.

	Total sites	Productive sites	*Species occurrences
Lotic Habitats			
Headwater	10	4	4
Non-Headwater	17	0	0
Sidechannels & Backwaters	3	3	12
Creeks	10	1	1
Total Lotic	40	8	17
Lentic Habitats			
Ponds & Oxbows	2	2	2
Reservoirs	4	2	2
Total Lentic	6	4	4
All Habitats			
Survey Total	46	12	21

* Species occurrences are the sum of the number of species at each site.

Although these results might suggest a sampling bias in the methods, their similarity to results reported for adjacent river basins tends to discredit such a conclusion. In the Elkhorn River, immediately south of the eastern portion of the study area, mussels are reported from headwater reaches but absent in lower reaches (Clausen and Havlik 1994, Hoke 1994b). West of the Elkhorn Basin, the main channels of the rivers of the Loup System are devoid of mussels (Hoke 1994a, 1999). These results are also supported by similar findings in the Niobrara River (Freeman and Perkins 1997).

Creeks were nearly as unproductive as habitats in the main river channels. These environments included 10 of the 46 sites sampled, but yielded only one productive locale, site 25 on Boardman Creek, and a single species occurrence, a fragment of a unionid shell. Mussels were reported in two other creeks, but were not recovered from either. The only identified species collected from a creek is *Pyganodon grandis* (see Freeman and Perkins 1997). The infrequency of unionids in creeks may be a reflection of limited access. Creeks are the most productive habitats for unionids in the adjacent Elkhorn and Loup basins (Hoke 1994b, 1999).

Lentic habitats comprised 13% of all collection sites but produced a third of all productive sites and 19% of all species occurrences. Bivalves were anecdotally reported at both of the unproductive lentic sites. In the interior of the study area, lentic habitats were occupied by *Pyganodon grandis*. In fact, with only two exceptions, these habitats defined the distribution of *P. grandis* in the region. The two exceptions were Minnichaduza Creek immediately below a dam (Freeman and Perkins, 1997), and one backwater along the lower Niobrara.

Explanation of unionid distributions

A number of environmental parameters greatly influence the distribution of bivalve molluscs in the study area. The most important is the presence of stable subsubstrates. Unstable sandy substrates have long been known to be detrimental to bivalves (Murray and Leonard 1962). Utilization of habitats by unionids in northern Nebraska was negatively correlated to the presence of shifting sandy substrates. Productive collection sites were characterized by slow currents or lentic conditions, and substrates consisted of mud and/or stable sand. Side channels and backwater areas protected from the full force of currents were the most productive habitats, both in species richness and abundance.

Bivalve distributions in northern Nebraska have also been adversely impacted by agricultural development through intensive grazing of livestock and water withdrawal for irrigation of cropland. Grazing appears to be the most widespread and significant of these for unionids. Grazing along streams often results in the destruction of riparian vegetation and resultant increases in soil erosion, fill up the deeper holes in streams, and eliminate habitat for fish. In streams flowing through sandy soil, intensive grazing may produce crumbling embankments, ultimately resulting in creation of streams that are significantly wider than their natural state. It seems likely that cattle crush unionid shells. Though bivalves were collected by the author from sites along the upper Niobrara River from which cattle had been denied entry, similar sites accessible to cattle were devoid of unionids. Because ranching is the predominant economic activity throughout much of the region, it is not surprising that reaches of streams protected from disturbance by domestic livestock are relatively rare. Grazing has been reported as a problem for unionids in Nebraska and northwestern Kansas (Hoke 1994b, 1995, 1997).

The adverse impacts of subsurface water removal are largely limited to Box Butte County, where dewatering was reported in a number of creeks including Box Butte Creek. This is the only county in the region exhibiting widespread and significant declines in groundwater levels (Mack *et al.* 1996b). In 1995, Box Butte County contained the greatest number of registered irrigation wells of any county in the western half of the study region (Mack *et al.* 1996a), and the related subsurface water withdrawals are probably the reason for the dewatering of Box Butte Creek, as well as the drastic lowering of water tables in the county. Possible evidence of the impact on unionids of surface water withdrawal was obtained at site 36 located about 34 km east of Box Butte Reservoir. At that site, the only specimens collected were weathered shells, many of which were broken. Because this reach of the river is subject to dewatering due to upstream withdrawals to an irrigation canal, it seems likely that these withdrawals may be responsible for the extirpation of former populations now represented solely by weathered shells.

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The late Bob Thomas, former Fisheries Chief, Nebraska Game and Parks Commission, donated specimens collected from site 45 in 1973. Assistance with identification of unionids was provided by David H. Stansbery and G. Thomas Watters of the Museum of Biological Diversity, Ohio State University. Information on the size of stream basins was supplied by Rich Kern of the Nebraska Department of Natural Resources. The officers of the Nebraska Game and Parks Commission and South Dakota Department of Game, Fish and Parks provided valuable input on unionid resources in northern Nebraska and the surrounding region. The author is indebted to the following museums and their staffs for helpful assistance during visits to locate specimens from the study area: Academy of Natural Sciences, Philadelphia, PA; Carnegie Museum of Natural History, Pittsburgh, PA; Field Museum of Natural History, Chicago, IL; Illinois Biological Survey, Champaign, IL; Museum of Biological Diversity, Ohio State University, Columbus, OH; Museum of Zoology, University of Michigan, Ann Arbor, MI; Nebraska State Museum, Lincoln, NE; U. S. National Museum, Washington, D. C.; and the University of Colorado Museum of Natural History, Boulder, CO. The editor and the two reviewers are thanked for their useful comments and critique of the initial manuscript.

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A new species of *Sonorella* (Pulmonata: Helminthoglyptidae) from western Texas

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Abstract: A new species of the genus *Sonorella* is described from the Hueco Mountains, Texas. It is one of the smallest species of *Sonorella* and its locality extends the northeastern known boundary of the genus.

Key Words: land snail, Helminthoglyptidae, Hueco Mountains

The Hueco Mountains are located in extreme western Texas along the border of El Paso and Hudspeth counties and continue a few kilometers northward into Otero County, New Mexico. In Texas, they extend approximately 32 km southward from the New Mexico border and have a maximum width of about 7 km. The mountains are bordered to the west by the Hueco Bolson, an intermontane basin of approximately 1,230 m in elevation, and to the east by the somewhat higher Diablo Plateau. In the northwestern part of the range, block-faulting has produced a prominent escarpment capped at about 1,645 m by a massive limestone formation called the "Rimrock" (USGS Hueco Tanks 7.5 min. quad. 1955). The Rimrock is formally named Hueco Canyon Limestone and is of early Permian age. It is approximately 150 m thick, thus forming a salient feature visible from far to the west in the Hueco Bolson (Fig. 1). The limestone disintegrates to produce, in some places, streams of talus down the west-facing mountainside below. Such talus is scattered along the Rimrock escarpment, extending from New Mexico for approximately 8 km south and southeast to north of Hueco Tanks State Historical Park. Because these talus spills face the west or southwest, they are exposed to the heat of the afternoon sun most days of the year. It is only in these talus accumulations that the new species of *Sonorella* Pilsbry, 1900, described herein, has been found. *Gastrocopta pellucida* (Pfeiffer, 1841) and *Metastoma roemeri* (Pfeiffer, 1848) also occur here (A. Metcalf, pers. obs.).

The land snail genus *Sonorella* contains over 100 described taxa that inhabit mountain ranges in the southwestern US states of Arizona, New Mexico, and Texas, as well as the northern Mexican states of Sonora and Chihuahua. Species of *Sonorella* are relatively large snails with shell diameters of approximately 13 to 30 mm. They constitute a major component of the molluscan fauna of this region. Only two species, *Sonorella orientis* Pilsbry, 1936, and *Sonorella metcalfi* Miller, 1976, have been described from Texas, the eastern edge of the range of the genus.

MATERIALS AND METHODS

Snails were collected in the field by the junior author (along with T. J. Dillon on one occasion). Three living specimens were drowned and their bodies were removed from their shells. Their reproductive systems were dissected free of the other organs, stained with Delafield Hematoxylin and Eosin B, and mounted on slides by LHG in the manner originally described by Gregg (1959) and later revised by Naranjo-García (1989).

Abbreviations of institutions cited in this paper are as follows: ANSP, Academy of Natural Sciences of Philadelphia; LACM, Natural History Museum of Los Angeles County; SBMNH, Santa Barbara Museum of Natural History; USNM, National Museum of Natural History - Smithsonian Institution; UTEP, University of Texas at El Paso.

SYSTEMATICS

Family HELMINTHOGLYPTIDAE Pilsbry, 1939
Clade Sonorellamorpha Roth, 1996
Clade Sonorellales Roth, 1996
Genus *Sonorella* Pilsbry, 1900

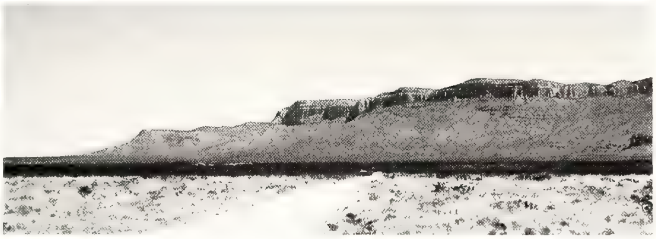

Figure 1. A view of the west-facing Rimrock escarpment of the northern Hueco Mountains, Texas, from the Hueco Bolson. Photograph by ALM.

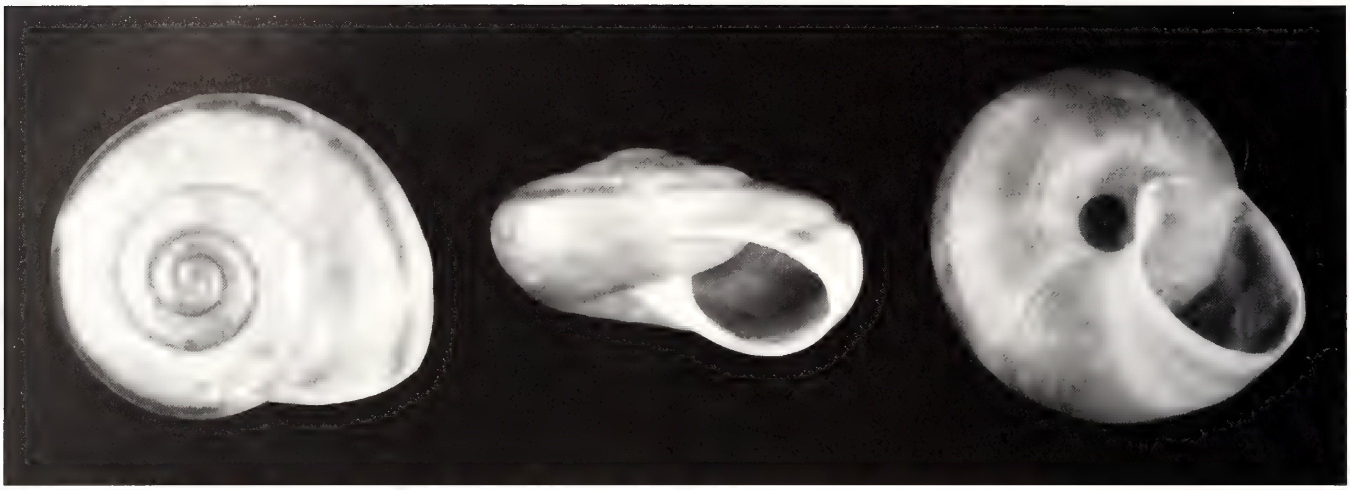

Figure 2. *Sonorella huecoensis* Gilbertson and Metcalf, sp. nov., holotype: apical view (left), apertural view (middle), umbilical view (right). LACM 2965 (exUTEP 12313). Shell 14.0 × 7.5 mm.

Sonorella huecoensis Gilbertson and Metcalf, sp. nov.
(Figs. 2-4)

Diagnosis

A very small *Sonorella* with a glossy, depressed shell; embryonic whorls exhibit very fine, radial, incised grooves. Male genitalia have a slender, smooth, cylindric to subclavate, bluntly-rounded verge and a penial retractor muscle inserting on epiphallus above apex of penis.

Description of holotype (LACM 2965, ex UTEP 12313)

Shell very small for genus (diameter 14.0, height 7.5 mm), umbilicate, depressed, heliciform, glossy, light tan, moderately thin, with a light brown spiral band on upper part of rounded shoulder of body whorl and 0.3 of penultimate whorl (Fig. 2). Umbilicus about one-seventh of diameter of shell (2.0 mm), slightly covered by reflected columellar lip. Whorls 4.4, moderately convex. Embryonic shell of 1.7 whorls very glossy and translucent with numerous, very fine, incised, somewhat anastomosing and interrupted radial grooves (producing a ripple effect) after smooth apex; threads and granules lacking. Post-embryonic whorls exhibit light growth wrinkles. Aperture oblique, rounded, slightly wider than high, margins converging. Peristome simple except for reflection of columellar lip.

Paratypes

Twenty-two representative paratypes from three collection lots (UTEP 4730, 11956, 12313) ranged from 12.8 to 15.2 mm in diameter ($\bar{x} = 13.9$) and from 6.6 to 8.0 mm in height ($\bar{x} = 7.3$) (Table 1). Several paratypes have a slightly thickened ventral margin of peristome. Many shells, including numerous unmeasured specimens, show effects of weathering and hence are not as glossy as holotype. Some shells exhibit weak, spirally descending threads and scattered, minute granules in addition to radial ripples on the embryonic whorls (Fig. 3).

Description of reproductive anatomy (Fig. 4, Table 2; LACM 2966): Description based on a mature, stained, slide-mounted paratype. Albumen gland and uterus typical in appearance; vagina approximately same length as penis. Spermathecal duct relatively long, unbranched; spermatheca small, round. Penis small, slender (about 0.4 mm diameter), internal wall of apical region with numerous, finely serrated rings (presumably glandular). Verge nearly one-half length of penis, elongate, slender, smooth, sub clavate, and surrounded by rather capacious lumen of penis. Penial sheath enveloping most of lower penis (following a slight medial constriction). Epiphallus about same length as penis; proximal region following vas deferens somewhat enlarged and attached to penial sheath by band of connective tissue. Penial retractor muscle long, thin, inserting on slender distal region of epiphallus above apex of penis. Epiphallic cecum (flagellum) very short, moderately thick, detached.

Variation of additional paratypes (Table 2, LACM 2966). Two additional slide-mounted specimens are very similar in appearance. However, both are somewhat smaller over-all and exhibit shorter verges (approximately one-third length of penis) that are cylindrical (not subclavate) in shape. One specimen clearly shows seminal duct opening centrally on bluntly rounded tip of verge.

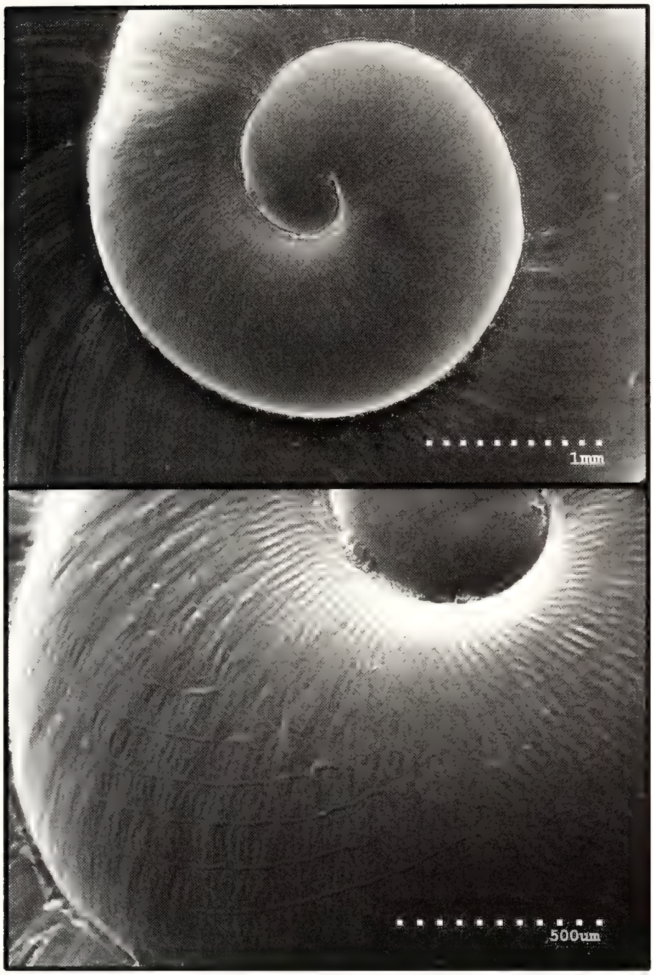

Figure 3. Scanning electron micrographs of the apical whorls of *Sonorella huecoensis* Gilbertson and Metcalf, sp. nov., paratype. Upper photo: apical view. Lower photo: oblique view.

Type locality. U.S.A., Texas, El Paso County, Hueco Mountains, 31°58′24″N; 106°02′54″W, limestone talus on west-facing escarpment below the Rimrock. Elevation 1,493 m. The holotype and 12 paratypes were collected by ALM on 2 November 1987 (UTEP 12313). Ninety-two paratypes were previously collected by ALM at this site on 17 January 1976 (UTEP 4730). Additional paratypes were collected at a similar, nearby site, 31°59′13″N; 106°02′47″W, 12 August 1989 by Timothy J. Dillon and ALM (UTEP 11956). This locality extends a section of the northeastern known boundary of the genus *Sonorella* and of the family Helminthoglyptidae, by approximately 50 km.

Freehling (1976) summarized climatic conditions for the southern Hueco Mountains, noting that average annual precipitation was 20.23 cm. Average midday relative humidity ranged from 21% in April to 39% in July (the peak month of the monsoonal rainy season) and 42% in January. The mean monthly temperature was 17.4°C with an average daily minimum in January of -1.0°C and an average daily maximum in July of 34.8°C.

Disposition of Types. Holotype: LACM 2965 - exUTEP 12313. Paratypes: ANSP 410946 - exUTEP 12313 (3); LACM 2967 - exUTEP 4730 (21), 2968 - exUTEP 11956 (8), three slides of reproductive anatomies 2966 - exUTEP 12313; SBMNH 348127 - exUTEP 12313 (3); USNM 1013148 - exUTEP 12313 (4); UTEP 4730 (76), 11956 (26).

Etymology. This species is named for the Hueco Mountains where it lives. For purposes where a common name is useful, the term "Hueco Mountains talus snail" is proposed.

DISCUSSION

Sonorella huecoensis is one of the smallest known species of *Sonorella*. Its small size may be an adaptation to the sea-

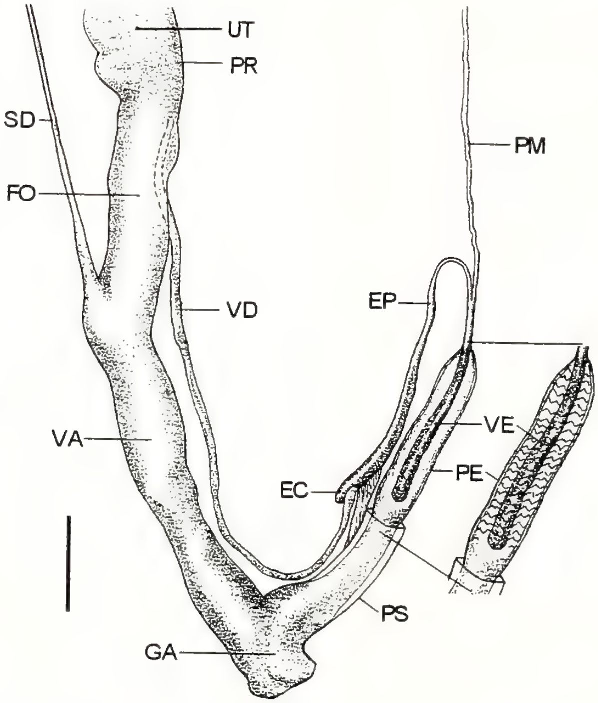

Figure 4. Diagram of the basal organs of a slide-mounted reproductive system of *Sonorella huecoensis* Gilbertson and Metcalf, sp. nov., paratype. Enlargement of proximal region of penis illustrates internal folds. The band of connective tissue between the penial sheath and the epiphallus has been artistically reconstructed. Abbreviations: EC, epiphallic cecum; EP, epiphallus; FO, free oviduct; GA, genital atrium; SD, spermathecal duct; PE, penis; PM, penial retractor muscle; PR, prostate gland; PS, penial sheath; UT, uterus; VA, vagina; VD, vas deferens; VE, verge. LACM 2966 (exUTEP 12313). Scale bar = 1 mm.

Table 1. Measurements of selected representative paratypes of *Sonorella huecoensis* Gilbertson and Metcalf, sp. nov., from three collection lots (diameter × height) in mm. See section on type locality for collection data.

*exUTEP 4730	**exUTEP 11956	#exUTEP 12313
14.1 × 7.0	14.6 × 7.9	15.2 × 7.6
14.0 × 7.5	14.3 × 7.5	14.6 × 8.0
14.0 × 7.3	14.1 × 7.5	14.2 × 7.2
14.0 × 7.3	14.0 × 7.4	14.0 × 7.2
14.0 × 7.1	14.0 × 7.5	13.8 × 7.2
13.1 × 7.2	13.9 × 7.4	13.7 × 7.0
x̄ = 13.8 × 7.2	x̄ 14.2 × 7.5	13.6 × 7.0
		13.4 × 6.7
		13.1 × 6.8
		12.8 × 6.6
		x̄ = 13.8 × 7.1

* LACM 2967

** LACM 2968

# ANSP 410946 (3); SBMNH 348127 (3); USNM 1013148 (4, including shells with largest and smallest diameters)

sonally hot, arid conditions of its west-facing rocky habitat. A similar tendency toward reduced size in helminthoglyptids that survive in highly stressful climatic conditions may be noted in *Sonorella micra* Pilsbry and Ferriss, 1910; *Maricopella allynsmithi* (Gregg and Miller, 1969); most species of *Eremarionta* Pilsbry, 1913; and others.

Species of *Sonorella* are usually found on volcanic rock, rarely on limestone (Pilsbry 1939: 268); therefore this new species is atypical for the genus. Another calcicolous *Sonorella* is *S. hachitana hachitana* (Dall, 1896) from neighboring southwestern New Mexico.

Other geographically proximate congenerics of *Sonorella huecoensis* are assigned to the *Sonorella hachitana* (Dall, 1896) "Group" or "Complex" (see Bequaert and Miller 1973:111; Metcalf and Smartt 1997:61-62; Miller 1968:14; Pilsbry 1939:273; Roth 1996:25,32). Unlike the new species, they are large snails with shell diameters in the 18-30 mm range. Their male genitalia are noticeably larger and typically exhibit an elongate, slender, pointed (*i.e.* acicular) verge that is variously sculptured (annulated, corrugated, or serrated). However, *S. huecoensis* shares several shell and genital features with *Sonorella metcalfi.*

Sonorella metcalfi is described from the Franklin and southern Organ Mountains, located over 50 km westerly across the Hueco Bolson, in Texas and New Mexico, respectively. This species is somewhat atypical of the *Sonorella hachitana* Group by having a smooth, bluntly rounded verge that is slightly less than half the length of its penis. Its verge, the shape of its epiphallus, and the insertion point of the penial retractor muscle on the epiphallus, resemble those of *S. huecoensis.* Its shell, while much larger (holotype diameter 19.8 mm; Miller 1976), resembles that of the new species in shape, color, and apical sculpture. Nevertheless, *S. metcalfi* and *S. huecoensis* are reproductively isolated from each other by a significant disparity in the sizes of their genitalia (*metcalfi* organs are over three times larger that the organs of *huecoensis;* Table 2). In addition, these two species are ecologically separated by the Hueco Bolson, which lacks suitable habitat for the survival of intergrading populations.

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Table 2. Genital measurements (length in mm) of *Sonorella huecoensis* Gilbertson and Metcalf, sp. nov. (three slide-mounted paratypes; LACM 2966) and *S. metcalfi* (holotype; Miller 1976).

Species	Penis	Verge	Penial sheath	Epiphallus	Vagina	Spermatheca/duct
S. huecoensis						
1. (Fig. 4)	3.7	1.7	1.1	4.0	3.8	17.8
2.	3.9	1.3	1.1	3.2	2.1	12.1
3.	3.5	1.4	1.2	3.6	2.9	—
x̄	3.7	1.5	1.1	3.6	2.9	15.0
S. metcalfi	13.0	6.0	6.5	14.0	10.0	—

(—) not available

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The glochidium and marsupium of *Castalia ambigua ambigua* Lamarck, 1819, from northern Brazil

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Abstract: As a contribution to the knowledge of the reproductive biology of the poorly known Amazonian freshwater mussel fauna, the present study describes the morphology of the glochidial valves and identifies the position of the marsupium in the inner demibranch of *Castalia ambigua ambigua* Lamarck, 1819, from the Irituia River, State of Pará, northern Brazil. The glochidium is sub-triangular in shape and has a mean length of 0.27 mm. Mean height was 0.26 mm, mean dorsal line length was 0.19 mm, and mean angle between the line that joins the ventral point to the middle of the hinge and the line from the ventral point perpendicular to the hinge was 0.95°. The larval valve is transparent and porous in appearance and has a triangular hook on the ventral margin of each valve. The adductor muscle is located in the central part of the valve. The marsupium is yellow in living animals and primarily occupies the posterior ventral portion of the inner demibranch.

Key words: freshwater mussel, glochidium, marsupium, Hyriidae, Brazil

The freshwater bivalves of the order Unionoida have complex life cycles in which the larvae are generally temporary parasites of fish (Cox 1969, Dillon 2000). The larvae are called glochidia in the Unionidae, Margaritiferidae, and Hyriidae; haustoria in the Mutelidae; and lasidia in the Mycetopodidae (Wächtler *et al.* 2001). The females produce eggs that are liberated into the suprabranchial chamber, where they are fertilized by sperm taken in through the incurrent siphon (Jirka and Neves 1992). The fertilized eggs descend by means of mucous threads to the water tubes of the gill, generally accumulating in one of the gill chambers, or marsupia (Mansur and Campos-Velho 1990), specialized for embryonic development.

In the Hyriidae, the marsupium always occurs in the inner demibranch (Wächtler *et al.* 2001), generally in a central position (Mansur and Campos-Velho 1990), but may vary depending on the species (Bonetto 1960a, Bonetto 1960b, Bonetto 1967, Ricci *et al.* 1990, Jupiter and Byrne 1997, Mansur and Campos-Velho 2000). Each fertilized egg develops via unequal cleavage into an embryo and then into a glochidium, which is characterized by two dorsally-articulated triangular or spherical valves (Mansur and Campos-Velho 1990). In the Hyriidae glochidium size varies from 50 μm (Jones *et al.* 1986) to 340 μm (Mansur and Silva 1999). Embryos of the Hyriidae are characterized by the absence of larval valves and the presence of a vitelline envelope, whereas glochidia have well-developed larval valves and a single large adductor muscle (Wächtler *et al.* 2001).

The glochidia were initially described as parasites of bivalves, but were eventually recognized as molluscan larval stages by Rathke in 1797 (Wächtler *et al.* 2001). In South America, the glochidium was observed for the first time by Lea in 1868 (Mansur and Silva 1999). Ortmann (1921) described in detail the larvae of the family Hyriidae and observed for the first time the absence of hooks in glochidia of *Diplodon hasemani* Ortmann, 1921; *Diplodon charruanus* d'Orbigny, 1835; and *Diplodon hildae* Ortmann, 1921. Bonetto (1960a, 1960b) described glochidia in the collections of the Field Museum of Natural History, Chicago, and the Carnegie Museum, Pittsburgh, highlighting the importance of glochidia in resolving systematic problems within the superfamily Unionoidea. Parodiz and Bonetto (1963) divided the Unionoida into two superfamilies based on larval type. Mansur and Campos-Velho (1990) presented techniques for the collection and study of glochidia from the South American Hyriidae and Mansur and Silva (1999) described the glochidia of five species of *Diplodon* (Hyriidae) from South America. The glochidia of some species of the tribe Castalini have been described by Bonetto (1960a, 1961), Martínez (1983), and Mansur and Campos-Velho (2000). In a recent review of larvae of the Unionoida (Wächtler *et al.* 2001), the different types of larvae within the order are described in detail.

Little is known about the freshwater mussel fauna of South America (Bogan 1993), especially that of the Amazon basin. To add to the knowledge of the reproductive biology of the fauna, the present study describes the morphology of the glochidial valves of *Castalia ambigua ambigua* Lamarck, 1819 and identifies the position of the marsupium in the inner demibranch. Such descriptions may eventually contribute to the resolution of systematic and ecological questions concerning Neotropical freshwater mussels.

METHODS

Bivalves were sampled between September 2000 and August 2001 from the Irituia River (47°26′5.9″W, 01°46′11.1″S), which is 45 km long and located in the northeast Amazon Basin in the State of Pará, Brazil. The climate is tropical with a mean air temperature of 25°C and a mean relative humidity of 85%. The rainy season occurs between January and June. Details of the simple random sampling scheme and the means of transport and processing of samples are described in Vale *et al.* (2004).

The demibranchs were dissected from the visceral mass and the inner demibranch was examined with a stereomicroscope. The position of the marsupium was determined by dividing the total area of the inner demibranch into four quadrants, with the aid of a cross-hair eyepiece: anterior dorsal (AD), anterior ventral (AV), posterior dorsal (PD), and posterior ventral (PV). The percentage of the marsupial area lying in each quadrant of the eyepiece was visually determined for each gravid female (n=50). The Kruskal-Wallis test was used to check for significant differences in the median percentage area of the marsupium occupying the four quadrants of the inner demibranch.

Each month, a sample of approximately 30 glochidia and embryos was examined with the stereomicroscope to describe the different stages of development and their principal structures, following Jones *et al.* (1986). Using an ocular micrometer, embryos were measured along the maximum diameter and glochidia were measured along their anterior-posterior axes. A sample of glochidia (n=100) was prepared for Scanning Electron Microscopy (SEM) and for light microscopy. The soft tissues of mature glochidia were removed with sodium hypochlorite (8 drops in 10 ml deionized water for 5 min). The valves were subsequently cleaned in deionized water in test tubes for 24 hr, with frequent changes of the fluid by means of a pipette. Some of these glochidia were dried on filter paper, mounted on stubs, sputter-coated with gold, and photographed using a Phillips XL30 scanning electron microscope. The remaining glochidia (n=46) were mounted in the horizontal position on permanent glass slides, and measurements of glochidial length (L), height (H), dorsal line length (DLL), and the angle (A) between the line that joins the ventral point to the middle of the hinge and the line from the ventral point perpendicular to the hinge (Fig. 1) were obtained following the procedures of Mansur and Campos-Velho (1990). Summary statistics were calculated from the morphometric data using the R package (Ihaka and Gentleman 1996).

RESULTS

A total of 389 glochidia were measured with the stereomicroscope. The mean anterior-posterior length of the larval valve (± sd) was 0.27 ± 0.01 mm. All glochidia had larval valves, a hook, and an adductor muscle. The glochidial valves had a sub-triangular shape (Fig. 1). The hook, which was located on the inner ventral margin of each valve, had a triangular form with a pointed end that was oriented dorsally toward the inside of the valve (Fig. 2). A protuberance occurred on the outer valve margin ventral to the hook (Figs. 2-3). The glochidial valves appeared porous and transparent (Fig. 4). The adductor muscle appeared as a wide bundle of

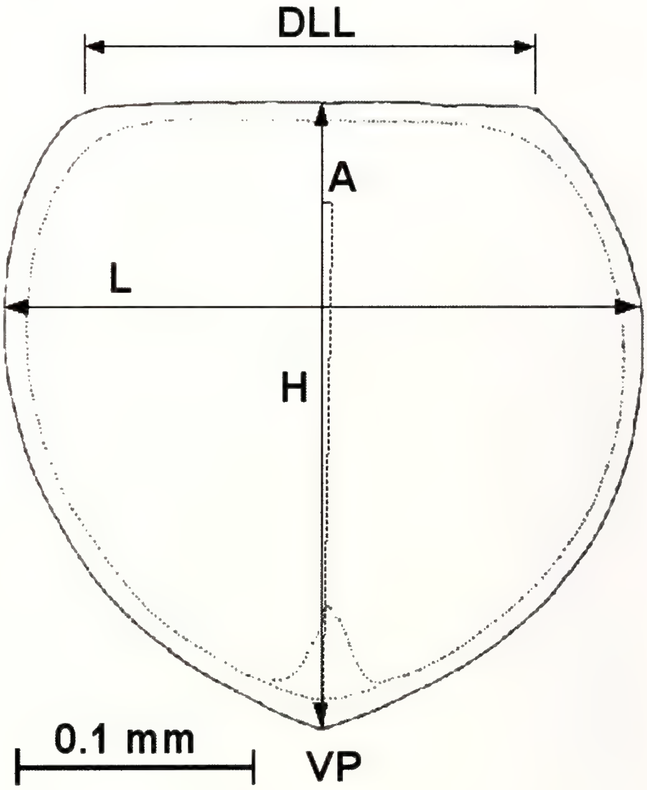

Figure 1. Schematic view of a glochidial valve of *Castalia ambigua ambigua* drawn from observations with the light microscope, showing the length (L), height (H), dorsal line length (DLL), and the angle (A) between the line that joins the ventral point (VP) to the middle of the hinge and the line from the ventral point perpendicular to the hinge.

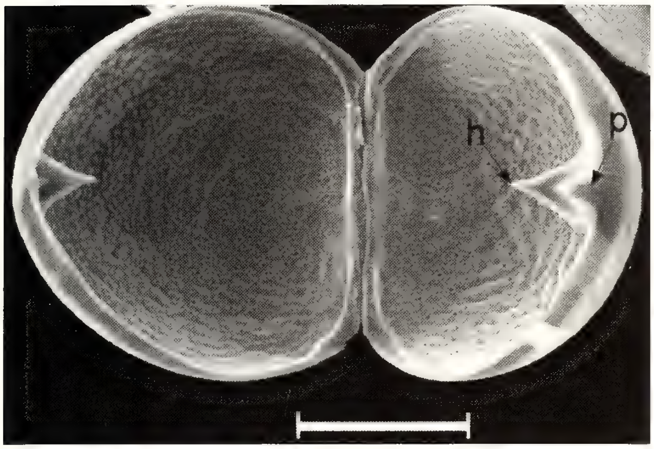

Figure 2. Scanning electron micrograph (SEM) of the internal view of the glochidial valves of *Castalia ambigua ambigua* showing the hinge line and triangular hook (h) located on the inner ventral margin of the valve. A protuberance (p) occurs on the outer valve margin, ventral to the hook. Scale bar = 100 µm.

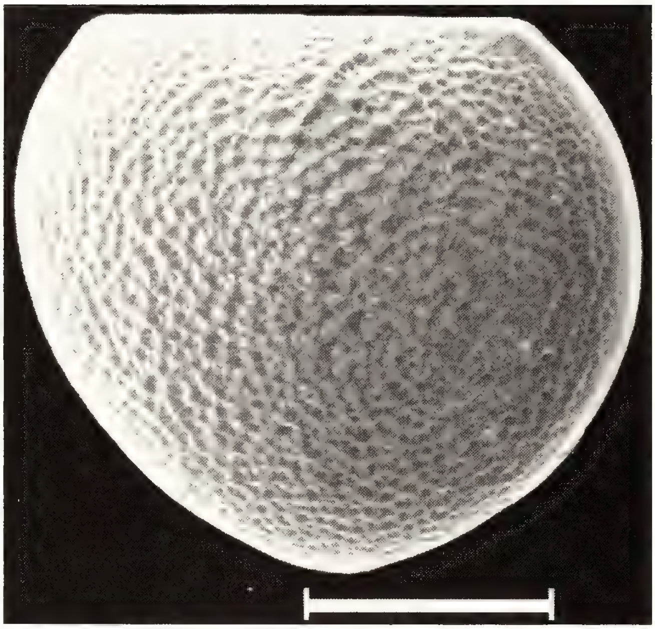

Figure 4. SEM of the external surface of a glochidial valve of *Castalia ambigua ambigua*. Scale bar = 100 µm.

dark brown fibers (in specimens preserved in 70% alcohol) extending between the central regions of each valve.

Table 1 summarizes the measurements obtained from the specimens (n = 46) examined with the light microscope. Glochidium mean length was 0.27 mm, mean height was 0.26 mm, mean length of dorsal line 0.19 mm, and mean angle (A) between the line that joined the ventral point to the middle of the hinge and the line from the ventral point perpendicular to the hinge was 0.95°. All measurements except A varied little (Table 1). A total of 355 embryos were measured with the light microscope. The mean length (± sd) was 0.22 ± 0.04 mm. Embryos were characterized by their round or oval shape, the absence of valves, and the presence of a vitelline envelope.

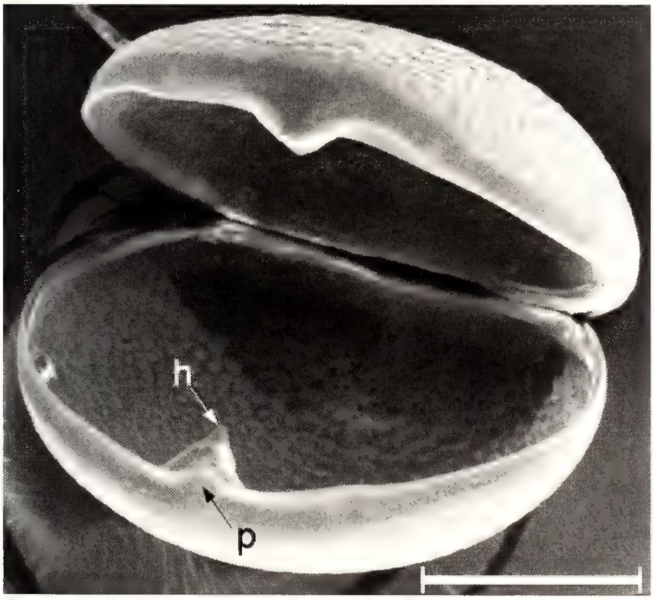

Figure 3. SEM of the ventro-lateral view of the gaping glochidial valves of *Castalia ambigua ambigua*, showing the hook (h) and protuberance (p). Scale bar = 100 µm.

The marsupium occupied the posterior ventral quadrant of the demibranch ($H = 128.75$, d.f. = 3, $p < 0.05$) to a significantly greater degree than it occupied the other three quadrants. On average, almost 75% of the marsupium

Table 1. Descriptive statistics of the morphology of the glochidium of *Castalia ambigua ambigua* from the Irituia River, Amazon Basin, Pará State, Brazil (n = 46).

Variable	Range	Mode	Mean	Standard error	Coefficient of variation (%)
Length	0.26–0.28	0.27	0.27	0.0008	0.0191
Height	0.24–0.28	0.26	0.26	0.0011	0.0274
DLL	0.18–0.20	0.19	0.19	0.0007	0.0249
A	0–2.5	1	0.95	0.1075	0.7621

DLL is the dorsal line length and A is the angle between the line that joins the ventral point to the middle of the hinge and the line from the ventral point perpendicular to the hinge. All measurements are in mm except A (degrees).

occupied the posterior ventral region of the inner demibranch. The marsupium appeared inflated and yellow in live specimens.

DISCUSSION

The size of the glochidium varies among members of the genus *Castalia* (Table 2). The mean length of the glochidium of *Castalia ambigua ambigua* (0.27 mm) was similar to values obtained by Bonetto (1961) for *Castalia psammoica* d'Orbigny, 1835 (0.26 mm-0.27 mm). However, the mean glochidial length of *C. a. ambigua* was greater than that of the majority of species of the tribe Castalini (Table 2). Of special interest is *Castalia acuticosta* Hupé, 1857, from the Guaropé River, Mato Grosso do Sul, Brazil, which Bonetto (1967) considered a synonym of *C. a. ambigua.* The glochidium of *C. acuticosta* is smaller than that of *C. a. ambigua,* suggesting a regional difference in glochidial size. The mean length of embryos of *C. a. ambigua* was similar to that reported for *Paxyodon syrmatophorus* Meuschen, 1781 in the Tocantins River, which measured 0.2 mm (Beasley *et al.* 2000).

For species of Hyriidae in South America and Australia, the largest glochidia belong to *Diplodon koseritzi* Clessin, 1888; *Hyridella drapeta* Iredale, 1934; and *Diplodon paulista* Ihering, 1893; whereas *Cucumerunio novaehollandiae* Gray, 1834 and *Hyridella australis* Linnaeus, 1758 have the smallest larvae.

Castalia ambigua ambigua has a relatively large-hooked glochidium. The hook of *C. a. ambigua* is similar to that described for *Callonaia duprei* Récluz, 1843 (Bonetto 1960a); *Castalia orinocensis* Morrison, 1843 (Martínez 1983); and *Castalia martensi* Ihering, 1891 (Mansur and Campos-Velho 2000). Large-hooked glochidia can attach to tougher tissues in the fins and to the scales, whereas smaller, hookless glochidia attach to softer tissues such as gill filaments (Davis and Fuller 1981). Although fish hosts of *C. a. ambigua* are unknown, from its glochidial size, we predict the presence of glochidia on the skin, scales, and fins of host fish.

Table 2. Mean lengths (mm) of glochidia from the family Hyriidae in South America and Australia.

Region and species	Mean length (mm)	Reference
South America		
Callonaia duprei Recluz, 1843	0.26	Bonetto 1960a
Castalia acuticosta Hupé, 1857	0.24	Ortmann 1921
Castalia ambigue multisulcata[1] Hupé 1857	0.23	Martínez 1983
Castalia inflata d'Orbigny, 1835	0.26	Bonetto 1961
Castalia martensi Ihering, 1891	0.25	Mansur and Campos-Velho 2000
Castalia neheringi Ihering, 1893	0.26	Ortmann 1921
Castalia psammoica d'Orbigny, 1835	0.26–0.27	Bonetto 1961
Diplodon berthae Ortmann, 1921	0.26	Mansur and Silva 1999
Diplodon charruanus d'Orbigny, 1835	0.28	Mansur and Silva 1999
Diplodon chilensis Grey, 1828	0.28	Parada *et al.* 1989
Diplodon decipiens Ortmann, 1921	0.3	Bonetto 1960b
Diplodon iheringi Simpson, 1900	0.28	Mansur and Silva 1999
Diplodon koseritzi Clessin, 1888	0.34	Mansur and Silva 1999
Diplodon martensi Ihering, 1893	0.29	Mansur 1999
Diplodon multistriatus Lea, 1831	0.28	Ricci *et al.* 1990, Bonetto 1960b
Diplodon paulista Ihering, 1883	0.32	Bonetto 1960b
Diplodon suavidicus Lea, 1856	0.31	Bonetto 1960b
Paxyodon alatus[2] Sowerby, 1869	0.25	Bonetto 1960a
Paxyodon syrmatophorus Meuschen, 1781	0.25	Beasley *et al.* 2000
Australia		
Alathyria profuga Gould, 1850	0.24	Jones *et al.* 1986
Cucumerunio novaehollandiae Grey, 1834	0.05	Jones *et al.* 1986
Hyridella australis Lamarck, 1819	0.07	Jones *et al.* 1986
Hyridella drapeta Iredale, 1934	0.33	Atkins 1979
Hyridella depressa Linnaeus, 1758	0.25	Jones *et al.* 1986

[1] Revalidated by Mansur (1991) as *Castalia orinocensis* Morrison, 1843.

[2] Considered by Haas (1969) to be a synonym of *Paxyodon syrmatophorus* Meuschen, 1781.

The sub-triangular shape and the porosity of the larval valves of the glochidium of *Castalia ambigua ambigua* resemble those of other species of Hyriidae (Bonetto 1960a, Bonetto and Ezcurra 1965, Atkins 1979, Martínez 1983, Jones *et al.* 1986, Mansur 1999, Mansur and Silva 1999, Jupiter and Byrne 1997, Mansur and Campos-Velho 2000). Pores in the larval valves may enhance nutrient flux and gas exchange (Pekkarinen and Englund 1995). The adductor muscle is similar to that described for *Diplodon martensi* Ihering, 1893 (Mansur 1999) but differs from that of *Anodonta cygnea* Linnaeus, 1758, which is located towards the anterior end of the glochidial valve (Wood 1974).

The marsupium in the Hyriidae is always in the inner demibranch (Parodiz and Bonetto 1963, Wächtler *et al.* 2001), although its location within the demibranch may vary considerably (Table 3). The posterior ventral position of the marsupium in the inner demibranch of

Table 3. Position of the marsupium in the inner demibranch in members of the family Hyriidae in South America and Australia. A = Anterior, P = Posterior, V = Ventral, M = Median.

Region and species	Position of marsupium	Reference
South America		
Castalia acuticosta Hupé, 1857	MPV	Ortmann 1921
Castalia martensi Ihering, 1891	MPV	Mansur and Campos-Velho 2000
Castalia undosa Martens, 1827	MPV	Ortmann 1921
Castalia neheringi Ihering, 1893	M	Ortmann 1921
Diplodon chilensis Grey, 1828	M, or whole demibranch	Parada *et al.* 1989
Diplodon decipiens Ortmann, 1921	M	Bonetto 1960b
Diplodon frenzeli Ihering, 1893	A	Bonetto 1960b
Diplodon multistriatus Lea, 1831	A, or AMV	Bonetto 1960b, Ricci *et al.* 1990
Diplodon paulista Ihering, 1883	M, or AM	Bonetto 1960b, Ortmann 1921
Diplodon suavidicus Lea, 1856	PV	Bonetto 1960b
Diplodon vicarius Ortmann, 1921	A	Bonetto 1960b
Paxyodon alatus (=*P. syrmatophorus*)	A	Bonetto 1960a
Australia		
Hyridella depressa Linnaeus, 1758	M	Jupiter and Byrne 1997

Castalia ambigua ambigua is similar to that described for several other Hyriidae (Table 3). The position of the marsupium has been found to vary between populations of *Diplodon chilensis* Grey, 1828 (Parada *et al.* 1989) and *Diplodon multistriatus* Lea, 1831 (Bonetto 1960b, Ricci *et al.* 1990), and between different individuals of *Diplodon paulista* (Bonetto 1960b), for example.

The growth and development of larvae inflates the marsupium of Hyriidae and gives it a yellow color in live specimens of *Castalia ambigua ambigua,* similar to that described for *Castalia martensi* (Mansur and Campos-Velho 2000). Avelar and Mendonça (1998) noted that the marsupium of *Diplodon rotundus gratus* Wagner, 1827, appeared dark brown when filled with mature glochidia, whereas when both embryos and glochidia were present, the marsupium appeared light brown. Similarly, the marsupia of *Paxyodon syrmatophorus* and *Triplodon corrugatus* Lamarck, 1819, vary between cream color and brown (C. R. Beasley, *personal observation*). Description of such variation in glochidium size and in marsupium position and color contributes to knowledge of the diversity, systematics, and ecology of the relatively poorly known Neotropical freshwater mussel fauna.

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Buoyant weight technique: Application to freshwater bivalves

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Abstract: A buoyant weight (W_b) method is described that makes use of bivalve weight underwater. Estimations of air fresh weight (W_f), total dry weight (W_d), shell weight (W_s), and tissue dry weight (W_o) were obtained from species-specific regression equations based on W_b. Standard regression curves were developed for two common freshwater bivalves, the Eastern Elliptio (*Elliptio complanata*) and the Asian Clam (*Corbicula fluminea*). Logarithmic estimations of W_b were also generated based on shell morphometric measurements. The estimates based on the technique showed that W_b of *E. complanata* and *C. fluminea* are linearly correlated with W_f, W_s, and W_o. In addition, shell volumes (length, height, and width measurements) of both species were shown to be good estimators of W_b. The buoyant weight technique is a simple and nonlethal means of generating different growth parameters in captive and wild freshwater mussels.

Key words: *Elliptio complanata, Corbicula fluminea,* shell volume, growth measurement

Growth measurements are a routine component of efforts to assess the health of aquatic species within surface waters (Bayne *et al.* 1979, Yap *et al.* 1998). Growth of free-ranging and laboratory populations of freshwater mussels have been monitored by measuring shell length, height, and width (DiDonato and Stiven 2001, Gatenby *et al.* 1997). Morphometric measurements have been a convenient means of monitoring growth because they estimate bivalve growth through increases in shell dimensions. In the past, relationships between shell length, height, and width were correlated with different dry weight parameters to approximate bivalve biomass (Isom 1971, Golightly and Kosinski 1981). However, overall bivalve health may not be accurately reflected by shell growth alone. Biomass parameters, through weight estimates, have also been used to estimate growth and net production of freshwater mussels (Isom 1971, Cameron *et al.* 1979). Unfortunately, some weight estimates (*e.g.* tissue dry weight) require the destruction of the whole animal. This poses a great dilemma for studies of rare and endangered freshwater mussels. As another method to estimate biomass, we suggest deriving an approximation based on a bivalve's weight under water that takes into account the overall increase in the organism – not just the increase in shell but also its increase in total organic matter.

Freshwater bivalves are calcareous invertebrates whose shell is composed of calcium carbonate ($CaCO_3$) crystals embedded within an organic matrix (McMahon and Bogan 2001). The shell organic matrix accounts for one-third to one-half of a mussel's total dry weight (shell and tissue organic matter) (McMahon and Bogan 2001). Growth of calcareous invertebrates can be monitored either through short-term and direct measurements of $^{45}Ca^{2+}$ and $^{14}CHO_3$ uptake or through long-term and indirect assessments of increases in shell weight (Darling and Wilbur 1993). By measuring the weight of calcareous organisms under water, one can indirectly assess the organism's rate of deposition of $CaCO_3$. Buoyant weight values can then be used to determine the weights of the shell and other components at a particular time. Buoyant weight measurements have been used extensively with great success to examine animals that have high calcite content, such as corals (Bak 1973, Davies 1989, Jokiel *et al.* 1978, Yap *et al.* 1998), marine molluscs (Nishii 1965, Palmer 1982), and barnacles (Darling and Wilbur 1993).

The buoyant weight technique estimates weight in air (fresh weight in air), total dry weight, shell dry weight, and tissue dry weight from species-specific regression equations. Accordingly, we obtained buoyant weight measurements from two common freshwater bivalve species in North Carolina to develop standard regression curves for total dry weight, shell dry weight, and tissue dry weight. We also determined the relationship of buoyant weight to shell morphometric measurements (length, height, and width) for comparison with values from the literature.

METHODS

Individuals of *Elliptio complanata* (Lightfoot, 1786) and *Corbicula fluminea* (Müller, 1774) were collected from Tar River, Swift Creek (Neuse Basin), and Mary's Creek (Cape Fear River Basin) in North Carolina, USA, by view bucket and snorkeling. Mussels were immediately transported in a water-filled cooler to the Freshwater Mussel Research Facility, located at the College of Veterinary Medicine, North Carolina State University. Mussels were cleaned with a light brush to remove dirt on the surfaces of the shells. Numbered tags (Hallprint Tags, Holden Hills, Australia) were fixed to the periostracum of each specimen using superglue. Mussels were then distributed between two closed recirculating systems and allowed to acclimatize for two days before further processing.

Each closed recirculating system held a maximum of 1700 L of temperature-controlled water and was composed of two stacked 500 L troughs, a 700 L reservoir/sump, and a trickle filter. Water in each system was pumped via a 1/2 hp centrifugal pump through the chiller unit to the head of the trickle filter. Water then descended through the trickle filter, which contained bio-surface elements and was captured in the upper trough. Through a 38 mm diameter stand pipe, water was gravity-fed from the upper to the lower trough and from the lower trough to the reservoir. From the reservoir, water flowed to the pump to complete the cycle.

Shell length (L), height (H), and width (W) of *Elliptio complanata* (n = 99) and *Corbicula fluminea* (n = 98) were measured. Then, each bivalve was weighed while immersed in water. Buoyant weight measurements were taken by attaching a weighing hanger under a top-loading analytical balance (Mettler Top-Loading Balance Model #BB240, Toledo, Ohio, sensitivity: 0.1 g) (Fig. 1). A plastic holding rack tied with monofilament fishing line was attached to the hanger and immersed in a plastic holding tub (~10 L) containing conditioned water. The balance was suspended over the plastic tub by a box constructed of plywood. Because water temperature affects density of water, water temperature was held constant and was monitored during measurements. Water volume was also maintained throughout the duration of the measurement. We controlled these parameters to maintain reproducible results of the experiment (Davies 1989, Jokiel *et al.* 1978).

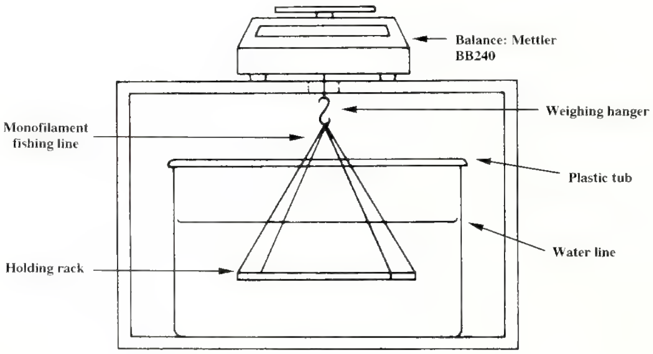

Figure 1. Schematic of the buoyant weight apparatus.

After the buoyant weight measurement, bivalves were rinsed with distilled water, blotted dry for one minute, and weighed in air to achieve fresh weight in air (W_f). Bivalve shells were opened with a blunt knife and adductor muscles were sliced to allow for easier opening of the shell. Mussel flesh was then removed with a blunt knife, transferred onto a pre-weighed aluminum baking cup (Reynold's aluminum baking cup), and dried to constant weight in a vacuum oven (70°C) to determine tissue dry weight (W_o). Shell dry weight (W_s) was obtained by air-drying mussel shells and then weighing each pair of valves using the Mettler balance. Total dry weight (W_d) was determined by adding W_s and W_o.

Shell density

To determine shell density, shell remains of sacrificed animals were immersed in 50:50 bleach solution overnight to remove the periostracum. Buoyant weights of bleach-treated shells (W_{b1}) were determined before air-drying them to constant weight (W_{d1}). Shell density was quantified and later compared with values from the literature to determine $CaCO_3$ composition. This comparison provided a means to check if what was being measured during buoyant weight was calcite. Shell density was calculated based on the density measurements published by Darling and Wilbur (1993):

$$\rho = W_{d1}\rho_w/W_{d1}\text{-}W_{b1}$$

where ρ is shell density, W_{d1} is shell constant weight after bleach treatment, ρ_w is water density, and W_{b1} is shell buoyant weight after bleach treatment.

Analysis

Weight parameters (W_b, W_f, W_o, W_s, and W_d) were log-transformed to satisfy assumptions of normality (Neter *et al.* 1996). Least-squares regression analysis was used to examine their relationship with buoyant weight (W_b) (Neter *et al.* 1996). Buoyant weight was assigned as the independent variable and the different weight parameters as the dependent variables. Log-transformed values of shell length, height, and weight were also compared with W_b and W_f using multiple regression analysis (Neter *et al.* 1996). Shell length, height, and width were assigned as the independent variables and W_b and W_f were designated as the dependent variables to allow for comparisons with the literature.

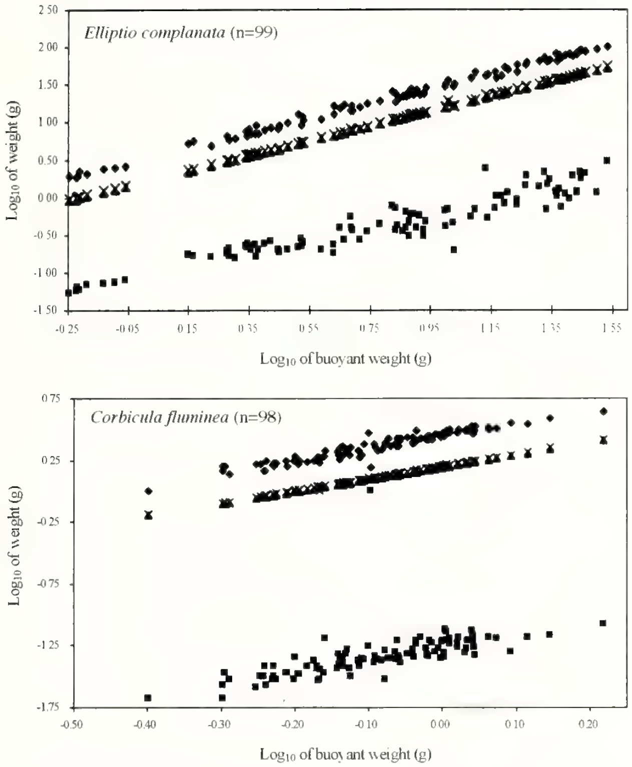

Figure 2. Relationship between the log-transformed parameters buoyant weight (W_b), fresh weight (W_f), organic tissue weight (W_o), shell weight (W_s), and total dry weight (W_d) of the freshwater bivalves *Elliptio complanata* (top) and *Corbicula fluminea* (bottom). W_f = ◆, W_o = ■, W_s = ▲, and W_d = ×.

RESULTS

Initial buoyant weight ranged from 0.57 to 40.99 g for individuals of *Elliptio complanata* and 0.31 to 1.65 g for individuals of *Corbicula fluminea*. The logarithmic regression equations for W_d, W_s, and W_f of both species identified strong linear relationships with W_b (Fig. 2). Shell weight (W_s) and total dry weight (W_d) had the highest correlation with W_b, whereas regression analysis between W_o and W_b resulted in a weaker relationship (Table 1).

Regression relationships between log-transformed values of length, height, and width and W_b revealed less variability among *Elliptio complanata* than *Corbicula fluminea*. However, the linear relationship between log-transformed buoyant weight and the length-height-width multiplier (LxHxW) was greatest for both *E. complanata* (98.5%) and *C. fluminea* (80.4%) (Table 1). Similarly, high regression coefficients were also generated from linear associations between log-transformed LxHxW and air weight for both species (Table 1). The t-statistic results from both log-

Table 1. Least squares linear regression of the Eastern Elliptio (*Elliptio complanata*) and Asian Clam (*Corbicula fluminea*) on (a) buoyant weight (W_b) vs. total dry weight (W_d), shell weight (W_s), organic tissue weight (W_o) and fresh air weight (W_f); (b) shell length (L), height (H), and width (W) and W_b; and (c) L, H, W and W_f.

Predictor	Coefficient	S. E.	t-statistic	R^2
Elliptio complanata				
(a) Logl0W_f				99.4
Slope	0.51	0.007	70.56	
$\mathrm{Log}_{10}W_b$	0.98	0.008	124.84	
$\mathrm{Log}_{10}W_o$				88.9
Slope	−1.03	0.030	−37.20	
$\mathrm{Log}_{10}W_b$	0.82	0.030	27.65	
$\mathrm{Log}_{10}W_s$				99.9
Slope	0.19	0.001	315.61	
$\mathrm{Log}_{10}W_b$	0.99	0.001	1527.64	
$\mathrm{Log}_{10}W_d$				99.9
Slope	0.22	0.001	198.66	
$\mathrm{Log}_{10}W_b$	0.99	0.001	841.36	
(b) $\mathrm{Log}_{10}W_b$				98.5
Slope	−3.72	0.092	−40.65	
$\mathrm{Log}_{10}L$	0.88	0.201	4.37	
$\mathrm{Log}_{10}H$	1.50	0.120	3.28	
$\mathrm{Log}_{10}W$	0.73	0.220	12.80	
(c) $\mathrm{Log}_{10}W_f$				96.3
Slope	−3.26	0.140	−22.68	
$\mathrm{Log}_{10}L$	1.05	0.320	3.32	
$\mathrm{Log}_{10}H$	0.89	0.350	2.56	
$\mathrm{Log}_{10}W$	1.09	0.180	5.95	
Corbicula fluminea				
(a) Logl0W_f				94.4
Slope	0.45	0.004	122.95	
$\mathrm{Log}_{10}W_b$	1.03	0.030	41.20	
$\mathrm{Log}_{10}W_o$				72.6
Slope	−1.27	0.008	−152.96	
$\mathrm{Log}_{10}W_b$	0.92	0.060	16.38	
$\mathrm{Log}_{10}W_s$				99.9
Slope	0.19	0.000	642.64	
$\mathrm{Log}_{10}W_b$	0.99	0.002	497.12	
$\mathrm{Log}_{10}W_d$				99.9
Slope	0.20	0.000	605.00	
$\mathrm{Log}_{10}W_b$	0.99	0.002	432.33	
(b) $\mathrm{Log}_{10}W_b$				80.4
Slope	−2.88	0.14	−20.25	
$\mathrm{Log}_{10}L$	0.76	0.18	4.16	
$\mathrm{Log}_{10}H$	1.16	0.14	2.65	
$\mathrm{Log}_{10}W$	0.48	0.18	8.12	
(c) $\mathrm{Log}_{10}W_f$				93.7
Slope	−2.66	0.09	−30.10	
$\mathrm{Log}_{10}L$	0.63	0.15	4.16	
$\mathrm{Log}_{10}H$	1.16	0.15	7.53	
$\mathrm{Log}_{10}W$	0.74	0.09	8.39	

transformed buoyant weight and air weight yielded the highest value from shell width in both species.

Shell densities of *Elliptio complanata* and *Corbicula fluminea* averaged 2.98 g/cm^3 (SD 0.05) (n = 34) and 3.07 g/cm^3 (SD 0.06) (n = 98), respectively.

DISCUSSION

The buoyant weight method provided a simple nonlethal means of monitoring growth in *Elliptio complanata* and *Corbicula fluminea* and should prove similarly effective for monitoring the growth of other species of freshwater bivalves. By quantifying changes in bivalve biomass, changes in buoyant weight indirectly represented growth of $CaCO_3$. The density of measured freshwater mussel shells clearly reflected $CaCO_3$ values in the literature (2.72 to 2.94 g/cm^3) (Lide 1999), suggesting that we were actually measuring shell calcite content. Apparently calcite was present, and not other carbonate forms such as aragonite, because temperature conditions in our system were such that calcite stability was encouraged more than aragonite formation (Berndt and Seyfried 1998). This demonstrates that buoyant weight measurements could effectively be used to monitor growth of freshwater bivalves. The buoyant weight procedure also provides a means of estimating parameters other than shell deposition. Through the calculation of regression equations, other growth parameters such as tissue weight can be generated to provide a more robust estimate of an animal's condition.

The logarithmic regression equations generated in this study provided a convenient way of predicting whole animal weight, tissue dry weight, and shell dry weight. The equations involving buoyant weight (W_b) and shell dry weight (W_s), dry tissue weight (W_d), and fresh weight (W_f) exhibited good fit ($R^2 > 94\%$). Shell weight exhibited the highest coefficient of determination in both species and accounted for 96% and 97% of the total dry weight for *Elliptio complanata* and *Corbicula fluminea*, respectively. Lower R^2 for tissue dry weight, compared to the other weight parameters, indicated tissue variation in the two species. These results agree with the findings of Hornbach *et al.* (1996), in which they described more variability in tissue parameters than shell parameters of *Amblema plicata* (Say, 1817).

Multiple regression analysis indicated that the "shell volumes" (LxHxW) for both *Elliptio complanata* and *Corbicula fluminea* were better predictors of W_b and W_f than were individual effects of shell length, height, and width. Isom (1971) also reported the same trends in predicting weight of *Pleurobema cordatum* (Rafinesque, 1820). As reported in this study, the high correlation between shell morphometric parameters and weight parameters (such as W_b and W_f) implies that if shell length, height, and width are the only measurements available, weight parameters of a freshwater bivalve can still be generated, provided regression equations have been established. Because shell morphometric measurements were shown to be linearly correlated with buoyant weight, we can deduce other weight parameters based on their linear relationship with buoyant weight. Therefore, the logarithmic regression equations we generated can provide estimates for different weight parameters without sacrificing individual animals.

Our results using multiple regression analyses between shell volume and buoyant weight corroborate the importance of relying on the combined measures of shell length, height, and width to estimate growth instead of shell length alone. Shell volume provided a more accurate representation of the shape of a freshwater bivalve. In this study, we reported that regardless of shell morphology, the relationship between shell volume and W_f or W_b was a better measure of growth than length alone.

In conclusion, the buoyant weight method provides a simple nonlethal means of monitoring freshwater bivalve biomass. Compared to previous estimates of biomass generated from morphometric measurements (Isom 1971, Cameron *et al.* 1979, Golightly and Kosinski 1981), the buoyant weight technique provides a more direct measure of biomass parameters.

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Effects of submersion and aerial exposure on clutches and hatchlings of *Pomacea canaliculata* (Gastropoda: Ampullariidae)

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Abstract: *Pomacea canaliculata* is a neotropical freshwater snail that has invaded many countries around the world, becoming a serious pest of rice and a menace to native snails. In southern Pampas (Argentina) from late spring to early fall females deposit aerial clutches composed of hundreds of calcareous eggs on emergent substrates. Local bodies of water frequently undergo fluctuations in water level, resulting in extensive submersion of clutches or dropping of hatchlings out of water. The effects of aerial exposure on hatchlings and of submersion on development were analyzed experimentally. The submersion of the egg masses of *P. canaliculata* increased the duration of development up to approximately 50% and caused a decrease of up to 32% in hatching rate. The calcareous capsule, large size, and dense packaging of eggs probably impede an adequate oxygen supply to embryos when submersed or may interfere with the hatching mechanism. The embryos were unable to develop completely and to hatch under water. Submersion frequently caused the disintegration of newly laid clutches and the premature hatching of eggs in late stages of development, resulting in hatchlings with underdeveloped bodies. Aerial exposure of hatchlings, under conditions similar to those occurring in the field in midsummer, killed 99% after only four days, irrespective of the presence or absence of moist soil. During droughts of several consecutive years recruitment could be seriously affected.

Key words: *Pomacea*, recruitment, embryonic development, egg masses, water level fluctuations

Pomacea canaliculata (Lamarck, 1822) is a neotropical freshwater snail that ranges from the southern Amazonas River (Brazil) to the southern Pampas (Argentina). Since 1980 it has been accidentally or deliberately introduced into southeastern Asia and the southern USA (Neck 1986, Wada 1997), becoming a serious pest of several crops, including rice and taro.

At the southern limit of its native area *Pomacea canaliculata* reproduces from late spring to early fall (Estebenet and Martín 2002). During the evening or overnight, females deposit aerial, conspicuous, pink egg masses with 30-1000 calcareous eggs (Cowie 2002) on a variety of substrates (such as plants, logs, and rocks). Clutch elevation depends on the availability of substrates, and can range from 1-5 cm when vertical substrates emerging from water are absent to 35 cm when suitable substrates (i.e., emergent plants, stream banks, trees) are present. The duration of embryonic development is highly variable (7-28 days) and inversely related to air temperature (Hylton-Scott 1934, Schnorbach 1995). Hatchlings fall into the water one by one or as a group when the clutch disintegrates.

Streams, rivulets, and ponds located in southern Pampas undergo remarkable fluctuations in water level (Martín *et al.* 2001), frequently resulting in extensive submersion of clutches or dropping of hatchlings beyond the receding water edge. The duration of the submersion or aerial exposure depends on the magnitude of water fluctuations and clutch elevation and position. Deleterious effects of clutch submersion (including arrested growth and increased embryonic mortalities) have been reported for the Florida apple snail *Pomacea paludosa* (Say, 1829) (Turner 1998). Although adults of *Pomacea* spp. are usually tolerant to exposure to air, their hatchlings seem to be far more susceptible to desiccation (Burky 1974, Mildward de Andrade 1981, Turner 1996, Darby and Percival 2000).

In this paper the effects of aerial exposure on survival of hatchlings and of submersion on the duration of development, clutch hatching rate, and the size of hatchlings are analyzed and their effects on recruitment are discussed.

MATERIALS AND METHODS

Individuals of *Pomacea canaliculata* were collected during summer 1999-2000 in the Curamalal stream (37°13′S, 62°08′W), Buenos Aires Province, Argentina, in the southern limit of the species' native distribution. Curamalal stream is a permanent current with quite hard and alkaline waters that undergoes significant fluctuations in water level (Martín and Estebenet 2002, Estebenet and Martín 2003).

Adult snails were transferred to a culture chamber (temperature of 25 ± 3°C, natural photoperiod of 10-15 h light/day) located 165 km south of the collecting site. Females and males were randomly selected to constitute sixty pairs that were maintained in 3 L aquaria filled to approximately 75% with tap water saturated with $CaCO_3$ with continuous aeration and lettuce *ad libitum*. After one week's acclimation the aquaria were monitored daily, the date of each new egg mass deposited on the aquarium walls was recorded, and the pair of snails transferred to a new aquarium.

Clutches were incubated out of water for different lengths of time in a culture chamber at room temperature ranging from 8.4°C to 29.5°C. Clutches were immersed in tap water (that is, the aquarium was totally filled) at different ages and were maintained submerged for variable lengths of time. Seven levels of age at immersion (0, 2, 4, 6, 8, 10, and 12 days) were combined with seven levels of duration of submersion (1, 3, 5, 7, 9, 11, and 13 days) in a factorial design with three replicates per cell. After the submersion period the egg masses were exposed to air until hatching. During the experiment 23 egg masses that were not immersed at all were used as controls. Another set of clutches of different ages (from 0 to 10 days) was immersed for 60 days to evaluate the effect of prolonged submersion.

The duration of embryonic development (DED, days), from date of egg laying to date of hatching of the first egg, was recorded for each clutch. The numbers of hatchlings and unhatched eggs were recorded for up to six days after the onset of hatching. Hatching rate (%) was calculated as the number of hatchlings divided by the total number of eggs in each clutch. Shell lengths (SL, mm) of ten hatchlings from each egg mass were measured to the nearest 0.01 mm with a stereoscopic microscope. Groups of 20-50 hatchlings from each egg mass were dried (80°C, 48 h), weighed to the nearest 0.1 mg, and reweighed after ignition (600°C, 4 h) to obtain total and ash dry weights (TDW and ADW, in mg, respectively) and relative ash content (AC, %).

Because DED of control egg masses varied with room temperature the relative duration of development (RDD) of each experimental egg mass was calculated as: $RDD = DED_{experimental}/DED_{control,}$ using the control egg masses deposited during the same week.

A total of 540 hatchlings (mean shell length 2.48 ± 0.15 mm, n = 30) from 5 egg masses that hatched in the same day in aquaria without water were placed in groups of 10 in 5-cm diameter containers filled with 4 cm of soil from the edge of a nearby stream. The containers were placed in a glasshouse in an unshaded location and under uncontrolled photoperiod and temperature. The soil of 27 containers was maintained under field capacity (moist treatment); that of the remaining containers was allowed to dry over the course of the experiment (dry treatment). Air temperature and relative humidity were continuously recorded. Three containers from each treatment were retrieved daily for nine days and the snails in each container were transferred to Petri dishes containing tap water. After a recovery period of 48 h at room temperature, snails that were not crawling were observed with a stereoscopic microscope to determine if they were alive (based on heart beating) and survivorship (%) was calculated.

To test for the effect of age at immersion and duration of submersion on the duration of development, hatching rate (arcsin square root transformed), and hatchling size, two-way Anovas were performed. Multiple comparison tests (LSD) were used to locate the differences between means.

RESULTS

The DEDs of control egg masses were highly dependent on air temperature: the duration of development lasted from 13 to 24 days for mean daily temperatures ranging from 14.9 to 25.5°C (averaged for the period between the spawning and hatching for each clutch) (Fig. 1A). Hatching rate of the control egg masses was 77.42% ± 7.76 (mean ± SD) and was independent of both DED and egg mass size (Fig. 1B,C). Hatchling shell length was highly variable, ranging from 1.95 mm to 2.99 mm and was not correlated with the size of the egg mass (Fig. 1D). Hatching was very synchronous within each clutch, lasting at most three days between the hatching of the first and last eggs.

Nine of the 21 clutches that were immersed on their first day (age at immersion = 0) detached from the aquarium wall and gradually lost their integrity so that the isolated eggs accumulated on the aquarium bottom. This precluded any incubation out of water after the completion of the assigned submersion time for these egg masses, so they were discarded. The development of the remaining twelve clutches was continued out of water as planned. On the other hand, all of the clutches that were immersed at an age of 12 days hatched during their first day of submersion, thus preventing the application of a duration of submersion longer than one day. These unexpected effects of submersion resulted in the loss of the two extreme age levels for the age at immersion factor of the original design, which was reduced to a 5 × 7 design. Two-way Anovas were performed on the data from this subset of treatments.

Duration of submersion and age at immersion significantly affected the hatching rate and RDD of the clutches; these effects were additive (Table 1). On the other hand, the weights of the hatchlings, ash content, and lengths were not affected by these factors.

The mean hatching rate of the egg masses that were 2-10 days old decreased steadily (about 3%/day) from 88% to 53% with an increase in duration of submersion (Fig. 2A). The effect of age at immersion on hatching rate was not so clear; the egg masses immersed at four and ten days of age showed lower values than those of the other treatments. The egg masses that were immersed at 12 days of age and hatched during the next day showed the highest hatching rate (83%).

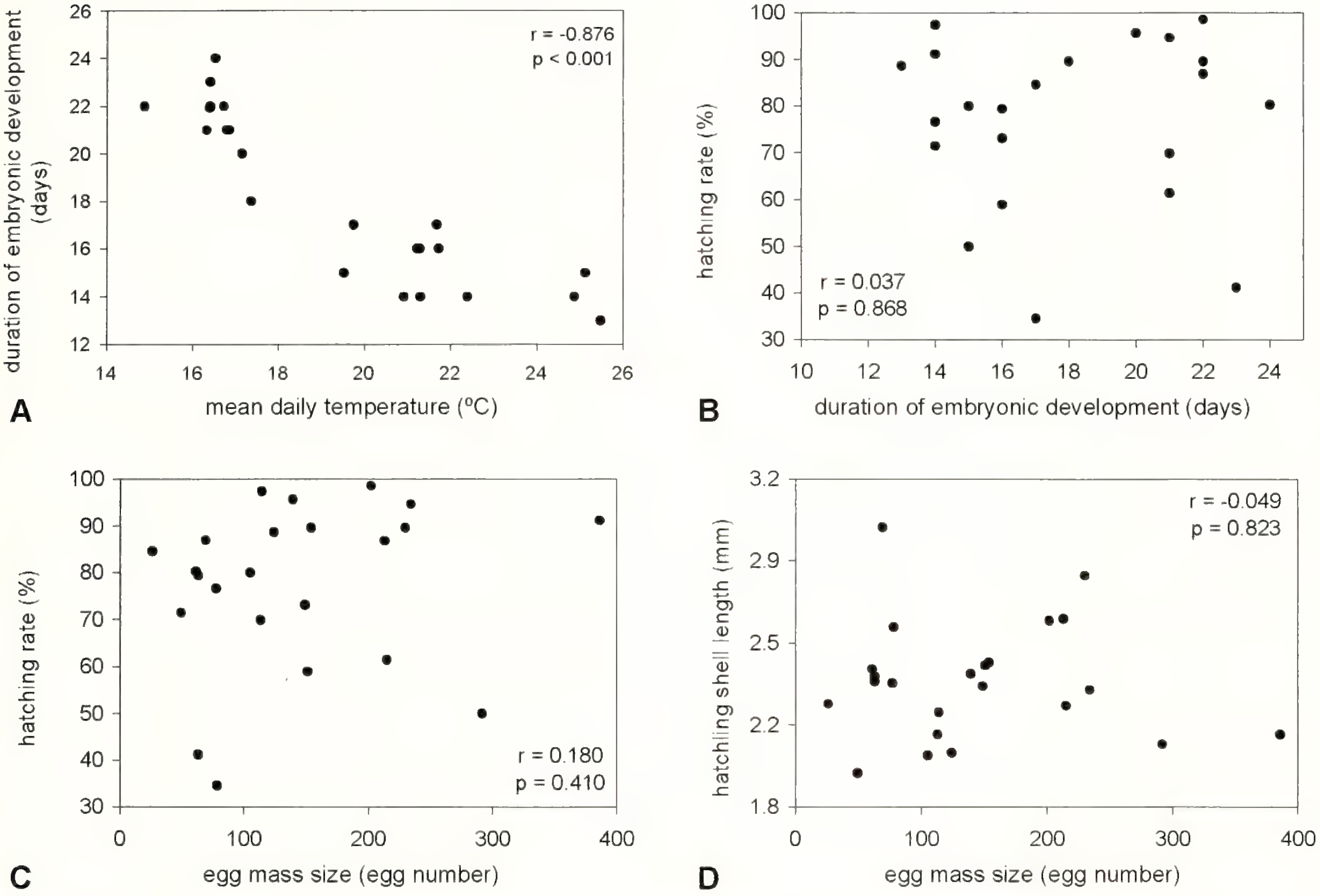

Figure 1. Scatterplots of selected variables measured on the control egg masses of *Pomacea canaliculata*. A, Duration of embryonic development *vs* mean daily temperature. B, Hatching rate *vs* duration of embryonic development. C, Hatching rate *vs* egg mass size. D, Hatchling shell length *vs* egg mass size.

On the other hand, the egg masses immersed the day they were spawned and that retained their integrity under water showed the lowest values (54%, Fig. 2B). Six of the nine egg masses that lost their integrity had durations of submersion longer than seven days.

The within-clutch synchronicity of hatching was not affected by submersion (that is, all eggs hatched in a period of three days). The RDD increased linearly (about 4.5%/day) with submersion duration (Fig. 3A), lengthening the development time up to 53% for the egg masses maintained under water for 13 days. However, submersion durations of one day did not prolong development relative to the controls. The RDDs of egg masses of all ages were affected by submersion (Fig. 3B). The egg masses immersed at 2 and 8 days of age showed the least and most delayed hatching, respectively. However, the RDD of 0 day old egg masses was higher than that of 2 day old ones, despite the fact that in this lot the clutches submerged during longer periods were those that tended to lose their integrity. The immersion of the 12-days-old egg masses caused a premature and massive hatching, reducing the whole development time by 14% relative to the controls.

All of the egg masses that were submerged for 60 days disintegrated and failed to hatch. These egg capsules were dissected to observe the degree of development of the embryos. After 60 days of submersion all of the observed embryos were dead (e.g., without ciliary or pedal movements), although there had been some growth before death. Only in the 0-day-old egg masses had the embryos not grown enough to be detected under 40× magnification.

Because the weights, ash content, and lengths of the hatchlings from egg masses immersed at different ages (between 2 and 10 days old) did not differ from each other (Table 1), they were pooled (n = 103) to compare them with the controls (n = 23). The latter were also compared with the clutches that were immersed when they were 12 days old

Table 1. Two-way Anovas for the effect of age at immersion and duration of submersion on hatching rate (%), relative duration of development (RDD, days), hatchling shell length (SL, mm), total dry weight (TDW, mg), ash dry weight (ADW, mg) and relative ash content (AC, %).

Variable	Source	M.S.	d.f.	F	P
Hatching rate	Age at immersion	4	0.12	3.137	0.02
	Duration of submersion	6	0.302	7.922	0.000
	Interaction	24	0.041	1.080	0.387
	Error	70	0.038		
RDD	Age at immersion	4	0.074	2.781	0.033
	Duration of submersion	6	0.546	20.373	0.000
	Interaction	24	0.029	1.065	0.404
	Error	70	0.027		
SL	Age at immersion	4	0.075	1.690	0.163
	Duration of submersion	6	0.027	0.599	0.730
	Interaction	24	0.029	0.658	0.873
	Error	68	0.044		
TDW	Age at immersion	4	0.341	1.741	0.151
	Duration of submersion	6	0.197	1.008	0.427
	Interaction	24	0.143	0.730	0.803
	Error	68	0.196		
ADW	Age at immersion	4	0.092	1.457	0.225
	Duration of submersion	6	0.083	1.315	0.262
	Interaction	24	0.069	1.093	0.375
	Error	68	0.063		
AC	Age at immersion	4	113.422	1.618	0.180
	Duration of submersion	6	63.895	0.912	0.492
	Interaction	24	100.367	1.432	0.126
	Error	68	70.079		

(n = 9). The SL, TDW, and ADW from control hatchlings were not different from those of clutches immersed when they were 2-10 days old (t = −1.32, p = 0.19; t = −0.51, p = 0.61; and t = −0.64, p = 0.52, respectively) nor to those 12 days old (t = 1.01, p = 0.32; t = 1.48, p = 0.15; and t = −0.03, p = 0.97, respectively) (Fig. 4). The ash content of hatchlings of 12-day-old clutches was significantly higher (t = −3.30, p = 0.003) than that of controls, but the latter was not different from the 2-10-day-old clutches (t = −0.32, p = 0.75).

Figure 5 shows the survivorship curves of snails maintained up to 9 days after hatching under dry or moist conditions and the corresponding variation in air temperature and relative humidity during the experimental period. The temperature varied daily between 16 and 27°C but showed two peaks of up to 39°C during the early part of the experiment. The survivorship of snails under moist conditions was very high during the first two days but decreased abruptly thereafter while that of those under dry conditions was seriously affected from the first days of aerial exposure. Five out of 30 snails exposed to air for 9 days survived under moist conditions. The hatchlings on dry soil had a dried body deeply withdrawn inside the shell; those on moist soil were partially buried with their apertures downwards and their opercula at the level of the apertural plane. Most snails from the moist treatment were alive when retrieved from the containers but died during the recovery period.

DISCUSSION

Submersion of the egg masses of *Pomacea canaliculata* affected both the hatching rate and the duration of development. Submersion increased the duration of development up to approximately 50% and caused a decrease of up to 32% in the hatching rate. Turner (1998) reported similar effects of submersion on embryonic growth and viability of the Florida apple snail *Pomacea paludosa*. The embryos of both species are unable to develop completely to hatching under water.

Calcareous egg capsules are common among land snails and slugs and clutch viability is highly dependent on their deposition in an adequately humid microenvironment (Tompa 1984, Heller 2001). Contrarily, the calcareous eggs of *Pomacea* spp. are truly cleidoic (i.e. exchanging only gases with the environment) and usually deposited on sites directly exposed to solar radiation and air currents (Perera and Walls 1996). *Pomacea urceus* (Müller, 1774), a species that incubates the eggs within the shell, is an exception, its embryos being highly susceptible to desiccation (Ramnarine 2003). The embryos of *Pomacea paludosa* (Turner 1998) and *Pomacea canaliculata* have evolved a physiological dependence on aerial incubation, being negatively affected by submersion. The high calcium concentrations reported in the perivitelline reserves of the eggs of these apple snails probably entails important physiological adaptations to avoid toxicity and osmotic problems (Turner and McCabe 1990, Catalán *et al.* 2002). The physiological stress provoked by submersion may be related to oxygen availability, solute losses, pH changes or osmotic problems.

The embryos of gastropods that lay aquatic eggs are usually affected by hypoxia, especially those in egg masses, where they compete strongly for oxygen (Booth 1995, Lee and Strathmann 1998), leading to longer development times or smaller sizes at hatching. The calcareous capsules of *Pomacea* spp., which appear to provide an efficient barrier against water loss during aerial incubation without impeding an adequate oxygen supply, presumably become a serious hindrance when submerged, because of the very much lower diffusion rates and concentrations of oxygen in water. The

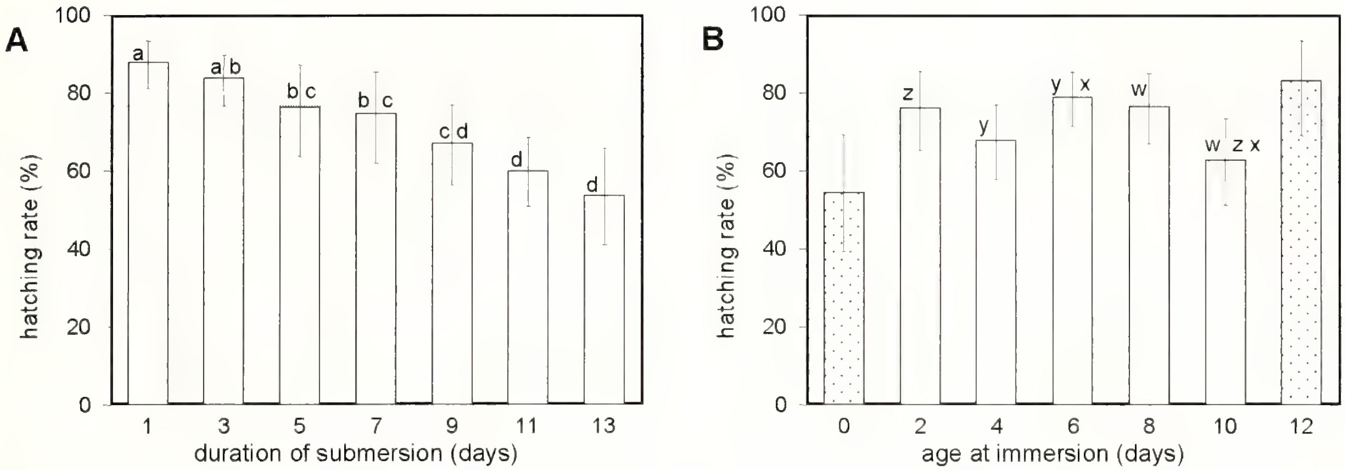

Figure 2. Hatching rate (mean and 95% CI) of immersed egg masses of *Pomacea canaliculata*. A, Effect of duration of submersion (means with the same letter were not significantly different, LSD, $p > 0.05$). B, Effect of age at immersion (means with the same letter were significantly different, LSD, $p < 0.05$). Dotted bars correspond to those levels of age at immersion not included in the two-way Anova.

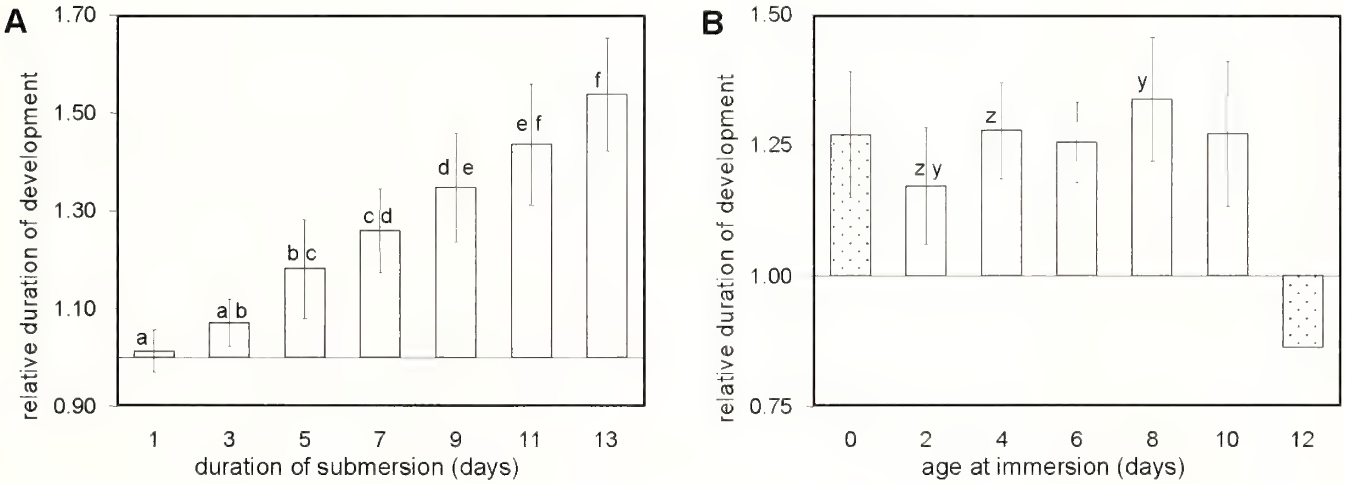

Figure 3. Relative duration of development (mean and 95% CI) of immersed egg masses of *Pomacea canaliculata*. A, Effect of duration of submersion (means with the same letter were not significantly different, LSD, $p > 0.05$). B, Effect of age at immersion (means with the same letter were significantly different, LSD, $p < 0.05$). Dotted bars correspond to those levels of age at immersion not included in the two-way Anova.

large size and dense packaging of eggs probably worsen this effect. The main energy store of the eggs of *Pomacea canaliculata* is carbohydrates, presumably allowing them to obtain energy either aerobically or anaerobically (Heras *et al.* 1998), although in the latter case at expense of lower growth rates. However, some evidence suggests that the effect of water is not solely due to oxygen availability. Schnorbach (1995) reported that embryonic growth of *P. canaliculata* is greatly inhibited by water condensation on the egg mass and Taylor *et al.* (1996) commented that repetitive wetting of the egg masses affects hatching and development negatively. The permeability of the egg capsule might be altered by the presence of free water, either decreasing oxygen input or increasing water loss. Fausto Filho (1965) reported that spawning in *Pomacea haustrum* (Reeve, 1856) decreases during the rainy season, despite prevailing high temperatures.

The embryos that were submerged for up to 13 days were able to develop under water and hatched after a period of aerial exposure. However, submersion was not tolerated for long periods, since a submersion of 60 days killed all the

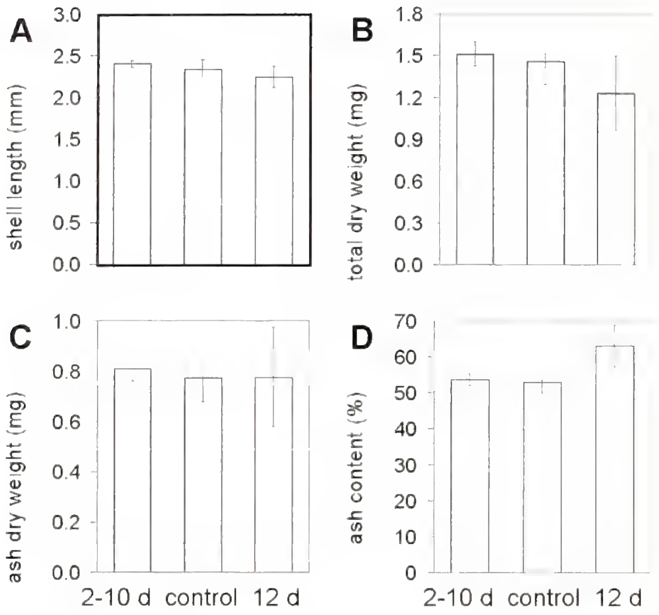

Figure 4. Means and 95% CI for variables measured on hatchlings of *Pomacea canaliculata* from egg masses immersed at different ages and for control egg masses. A, Shell length. B, Total dry weight. C, Ash dry weight. D, Ash content.

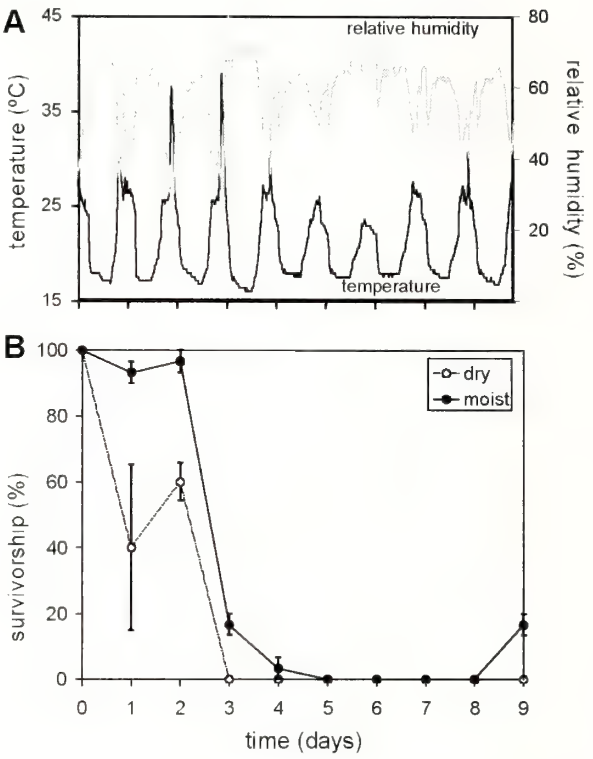

Figure 5. Effect of aerial exposure on hatchlings of *Pomacea canaliculata*. A, Temperature and relative humidity during the experiment. B, Survivorship (mean and 95% CI) on dry and moist soil.

embryos regardless of their age at immersion. The egg masses disintegrated before four weeks of submersion, which in the wild would impede an eventual aerial reexposure after a decrease in water level. In *Pomacea paludosa* a submersion period of only 20 days resulted in mortalities of about 85% for 1-day-old egg masses (Turner 1998).

The age at which egg masses were immersed affected the hatching rate and duration of development, but its effect was not so important and the pattern was not so clear as for the duration of submersion. The 0-day-old egg masses were especially sensitive to submersion: many disintegrated, especially after several days under water, and those that retained their integrity showed the lowest hatching rate. Irreversible changes during drying on the first day seemed to occur in the jelly or mucus that cemented the eggs, because no older egg mass lost its integrity before thirteen days of submersion. In *Pomacea paludosa,* tolerance of embryos to submersion increases with age and the "jellied" egg masses (less than one day old) often detached from their substrate after submersion (Turner 1998). According to Andrews (1964) the calcareous fraction of the egg capsule precipitates within 24 h. of egg laying; submersion may affect its final structure.

Under aerial incubation, the eggs of *Pomacea canaliculata* and *Pomacea paludosa* hatch by fracture of the calcareous capsule. An embryonic shell of a size equal to the egg diameter seems necessary for hatching (Estebenet and Cazzaniga 1993, Turner 1998). In 12-day-old egg masses of *P. canaliculata,* immersion induced a premature hatching without negatively affecting hatching rate. After only two hours of submersion the fracture of the first egg capsules was observed and the emergence of hatchlings began soon afterwards. The entire egg mass disintegrated before the completion of the first day of submersion. On the other hand, no massive, immediate hatching occurred in *P. paludosa* when late-stage egg masses were submersed (Turner 1998). The premature hatching under water of *P. canaliculata* probably indicates a critical weakening of the egg capsule from 10 days of aerial incubation onwards, since it did not occur in younger egg masses. Turner and McCabe (1990) suggested that the minor changes observed in the structure of the inner capsular layer during embryogenesis of *P. paludosa* were probably related to the hatching process. The pre-hatching weakening of the capsule in *P. canaliculata* seems to be more important than in the latter species, resulting in the implosion of the eggs when submerged. Presumably, this phenomenon would help some deeply located embryos to be freed from the remains of the egg mass, and thus could explain the high hatching rate of the 12-day-old egg masses.

Although no significant differences were observed in the lengths and weights of hatchlings from egg masses immersed at different ages, the shell lengths of the premature hatchlings were clearly shorter than the diameters of the eggs from which they emerged (pers. obs.). The great variation in egg size among clutches (Estebenet and Cazzaniga 1993) probably blurred the differences among treatments. Only the relative ash content of the premature hatchlings was significantly higher, probably indicating an underdeveloped body at the time of hatching. Although long term negative effects of premature hatching on growth and survival cannot be discarded, these hatchlings are readily able to perform the same activities as those born naturally. Turner (1998) observed that late embryos of *Pomacea paludosa* artificially liberated from their egg capsules were competent to assume an independent life.

Like other apple snails (Cowie 2002), adult *Pomacea canaliculata* are able to survive for several months out of water (d'Orbigny 1847, Mochida 1991, Schnorbach 1995). However, the resistance of the apple snail spat to aerial exposure is lower than in adults (Hurdle 1973, Burky 1974, Mildward de Andrade 1981, Turner 1996, Darby and Percival 2000). In our study, the combination of air exposure and high temperature peaks killed 99% of all hatchlings after only four days under conditions similar to those occurring in the field in midsummer. The hatchlings on dry soil died by dehydration, but those partially buried in moist soil probably died by a direct effect of temperature.

The reproductive season of *Pomacea canaliculata* in the southern limit of its native area runs from late spring (October) to early fall (April) (Estebenet and Martín 2002). The temperature variation during this period is similar to that experienced by our control egg masses and suggests that the duration of development could vary naturally between two and four weeks.

Rainfall in this semiarid area is highly seasonal (González Uriarte and Orioli 1998), resulting in marked fluctuations in the water level. The interannual variation in rainfall is also very high (Scian and Donnari 1997), resulting in successive inundations and droughts of several years of duration. The continuous rise of water level during inundations would submerse the egg masses long enough to kill the embryos. Even if water recedes before the death of the submersed embryos, development will be arrested, and under the low air temperatures at the end of the spawning period, would result in a very late hatching (April). This would increase the number of snails reaching winter and entering resting state (Estebenet and Martín 2002) with very small sizes, affecting their survival to the next spring. Watanabe *et al.* (2000) reported that overwintering mortalities of *Pomacea canaliculata* in drained paddy soil were very much higher for small snails.

During prolonged droughts most of the marginal fringe of rushes and cattails, the most common spawning substrate, will stand progressively beyond the shoreline, causing the dropping of hatchlings on exposed bottom and seriously affecting the recruitment during several reproductive seasons. In *Pomacea* spp. recruitment seems to be affected by drops in water level (Kushlan 1975, Donnay and Beissinger 1993) but it is not known if this is due to the aerial exposure of hatchlings or to harmful effects on other stages.

The detrimental effect of submersion on survival of embryos of the Florida apple snail *Pomacea paludosa* (Turner 1998) seems to be greater than the one reported here for *Pomacea canaliculata*. On the other hand, Darby and Percival (2000) reported survival times for hatchlings of *P. paludosa* out of water quite longer than those of *P. canaliculata*, although records close to those obtained here exist (Turner 1996). These differences are probably related to the different hydrological patterns in their respective habitats. In Florida, under a tropical climate with a dry season, the spawning of *P. paludosa* is concentrated mainly in April and May (Darby *et al.* 1999), coinciding with the last part of a period of decreasing water levels (Kushlan 1975), so the risk of egg submersion is low and that of aerial exposure of hatchlings high (Darby and Percival 2000). In the semiarid Southern Pampas, both risks are high and vary in an unpredictable way, probably resulting in a higher tolerance to submersion. On the other hand, the combination of high temperatures and very low relative humidities during the reproductive period probably overcomes any conceivable tolerance of hatchlings, so the selective pressures favoring a high resistance to aerial exposure are low.

Turner (1998) stressed the importance of the manipulation of water levels in wetlands to avoid negative effects of egg mass submersion on recruitment and hence ensure the conservation of *Pomacea paludosa*. On the other hand, the management of water levels to submerse egg masses or to provoke the aerial exposure of hatchlings could be used to control the population of pest apple snails in rice fields. In the case of *Pomacea canaliculata*, for the submersion of egg masses to be effective, a continuous rise (or at least repeated increases) in water level during most of the reproductive season would be required. These water regimes are hardly practical. On the other hand, repeated drainage of paddy fields to expose soil for short periods (less than four days) would kill most hatchlings and perhaps most of the youngest snails. Drainage of paddy fields after sowing or transplanting also immobilizes apple snails and reduces damage to rice seedlings (Litsinger and Estano 1993, Wada *et al.* 1999) and could enhance predation by fire ants on egg masses (Yusa 2001) and adults (Stevens *et al.* 1999).

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Sexual differentiation and size at first maturity of the invasive mussel *Perna viridis* (Linnaeus, 1758) (Mollusca: Mytilidae) at La Restinga Lagoon (Margarita Island, Venezuela)

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Abstract: The Asian green mussel *Perna viridis* was first recorded in the Americas in 1992 at Trinidad Island. In 1995 it was observed in the mainland coast of South America at Sucre State (Venezuela) and in 1998 at La Restinga Lagoon, Margarita Island, Venezuela. We studied the size at sexual differentiation, size at first maturity, and gametogenesis of *P. viridis* from La Restinga Lagoon using histological sections. One hundred specimens of *P. viridis* between 8 and 91.2 mm in total shell length were examined. No gonadal development was observed in individuals smaller than 18 mm. Sexual differentiation began at 20 mm with incipient follicular formation. Specimens larger than 25.0 mm had well-developed gonads at different stages of maturation. Size at first maturity was larger than previously reported at other localities. Because the lagunar mangrove system is permanently open to the sea, larval supply could come either from outside or inside the Lagoon, assuring the recruitment of new spat and the dispersal of the species. The introduction of this invasive species into the Lagoon has increased the available hard substrata for settlement of sessile invertebrates.

Key words: Invasive bivalves, size at sexual maturity, reproduction, Margarita Island, South America.

The Asian green mussel *Perna viridis* (Linnaeus, 1758) was first detected in the Americas at Trinidad Island in 1992 (Agard *et al.* 1992). In 1995, this species was reported from the South American mainland coast (northern Sucre State of Venezuela) (Penchaszadeh and Velez 1995, Rylander *et al.* 1996) and in 1998 at La Restinga Lagoon (Segnini de Bravo *et al.* 1998). Although in Asia *P. viridis* usually inhabits estuarine waters where salinity ranges between 27 and 33 ppt, it can tolerate salinity as low as 16 ppt (Sundaram and Shafee 1989). The optimal range of temperature for *P. viridis* in its native environment is 26–32°C (Sivalingam 1977). This bivalve generally occurs from the surface to 10 m depth and the presence of massive populations of juveniles has been recorded at depths between 2 and 3 m (Tan 1995, Cheong and Chen 1980). Like other mytilids, *P. viridis* is a dioecious species, with follicles covering most of the dorsal and ventral body surfaces and penetrating into the visceral mass and mantle. During gametogenesis the gonads are bright orange in females and creamy white in males (Walter 1982).

In February 2002 we observed specimens of *Perna viridis* attached to the roots of the red mangrove *Rhizophora mangle* at La Restinga, a tropical coastal lagoon located on Margarita Island, Venezuela. La Restinga is a slightly hypersaline lagoon, and considered a negative estuary because its salinity is always higher than that in the adjacent ocean (Scelzo 1999). In addition, the average water temperature in the Lagoon is higher than that in the outer coast, which is characterized by seasonal upwellings (Gómez 1999). At the Lagoon, the mytilid *Brachidontes exustus* (Linnaeus, 1758) coexists with *P. viridis*, but no colonies of the common open-sea mussel *Perna perna* (Linnaeus, 1758), a temperature-sensitive species (Hicks *et al.* 2001), have been found. The mangrove oyster *Crassostrea rhizophorae* (Guilding, 1828) is the most abundant invertebrate colonizing the mangrove roots. This bivalve acts as a substrate for the attachment of other molluscs such as *Crepidula aplysioides* Reeve, 1859 (Miloslavich *et al.* 2003).

To determine if this mussel population is gonadically active we studied sexual differentiation and first sexual maturity based on histological sections and following the maturity scale of Ingrao *et al.* (2001).

MATERIALS AND METHODS

Site description

La Restinga Lagoon National Park (64°2′-64°12′W, 10°90′-11°2′N) is located in Nueva Esparta State, Venezuela. It is bounded on the east by Margarita Island and on the west by the Peninsula de Macanao (Fig. 1). The Lagoon covers a total area of 25 km^2 and is characterized by an interconnected system of the red mangrove *Rhizophora mangle* and by muddy bottoms with patches of the seagrass *Thalassia testudinum*. La Restinga Lagoon is permanently

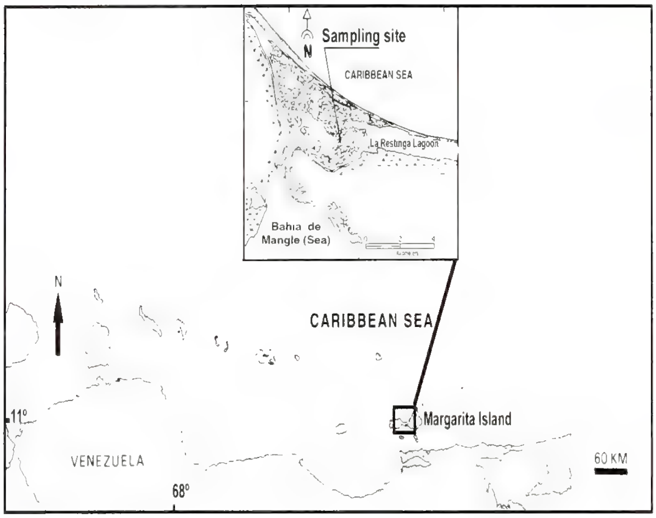

Figure 1. Map indicating the locality of collection of *Perna viridis* in La Restinga Lagoon, Isla Margarita, Venezuela.

open to the sea by a mouth of approximately 160 m in width and has a maximum depth of 5.6 m, decreasing towards the centre of the lagoon (Cervigón and Gómez 1986, Ramírez-Villaroel 1996). Marine diurnal tides produce strong currents at the mouth of the lagoon causing water circulation. These tides vary the water level into the lagoon by 30–50 cm, with a maximum peak of 90 cm in September and October (Gómez 1991). Salinity varies annually between 37.4–58.0 ppt at the Lagoon and between 36.0–37.8 ppt at the open sea nearby. The mean annual water temperature ranges between 27–40°C at the Lagoon (Scelzo 1999, Segnini de Bravo *et al* 1998), and between 20–24°C at the sea nearby, dropping down to 18°C in the open ocean just offshore (Gómez 1991).

Field and laboratory work

Two samples of 50 specimens (total 100) of *Perna viridis* were collected by hand from a boat from roots of the red mangrove (*Rizophora mangle*) at a depth of about 0.5 m at the same site in February and July 2002 in La Restinga Lagoon (Fig. 2). We tried to collect the full range of sizes in order to observe all the gonadal stages. Immediately upon collection, the whole soft body was removed from the shell, fixed in Bouin's fluid for 48 hours, and stored in 70% ethanol. The total shell length of each individual was measured using a caliper. Mantles were cut into square pieces of 3mm per side and prepared for light microscopic examination of the gonads with routine paraffin embedding, sectioning, and hematoxylin-eosin staining methods as described in Bigatti *et al.* (2001). Gonadal development of the 100 mussels was classified into five stages as follows:

Stage 0, neither sex nor gonads were differentiated, no follicles were developed; Stage I, follicles starting to develop, sex could not be determined; Stage II, active follicle and gamete development, genital material was dispersed throughout the body and mantle, in females oocytes accounted for at least 70% of the follicles, male cells maturing in the follicles as gametogenesis progressed; Stage III, gonads were ripe, gametes were mature and individuals were ready to spawn; Stage IV, spawning, the gametes were released. We considered females to be mature when their oocytes reached 50 µm in diameter, which was the maximum oocyte size recorded before spawning.

We used the parameters of Ingrao *et al.* (2001) for the shell length/age ratio recorded for Florida, USA, as the nearest locality with available information with similar water temperatures.

Slides were observed using a Zeiss Axiostar light microscope. Digital photographs were taken with a Sound Vision digital microscope camera Version 2.0 and plates were prepared using Adobe Photoshop® 5.5.

RESULTS

The mangrove roots were characterized by vertical zonation in community composition. The mangrove oyster *Crassostrea rhizophorae* dominated in the upper zone (down to 20–30 cm depth), *Brachidontes exustus* and unidentified sponges and hydrozoans dominated the middle zone, and *Perna viridis* dominated the lower zone. The shell surfaces of the mussels were colonized by invertebrates such as the gastropod *Crepidula aplysioides* (in very high densities, up to 200 individuals of different sizes attached to one mussel),

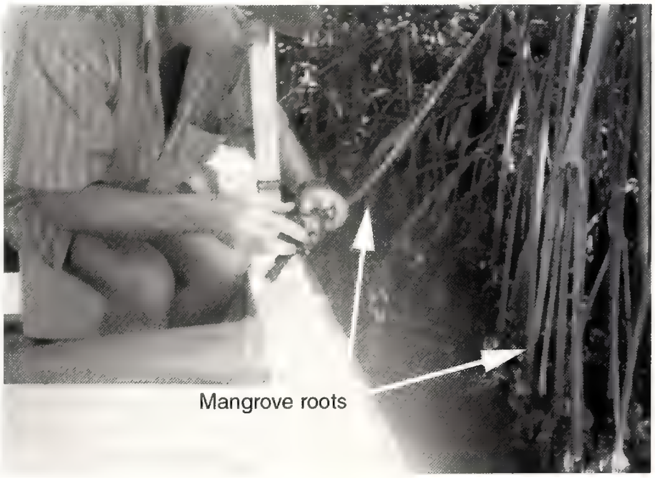

Figure 2. Red mangrove roots in La Restinga Lagoon, from where the material was collected.

barnacles, colonial bryozoans, tunicates, sponges, and polychaetes (Fig. 3).

The shell lengths of the collected mussels ranged between 8 mm and 91.2 mm for both months. No gonadal development was observed in individuals smaller than 18 mm (Stage 0). Sexual differentiation with incipient development of follicles started at a size of 20 mm (Stage I). Specimens larger than 25 mm had well-developed gonads and males and females were at different stages of maturation (Fig. 4). The average oocyte diameter for Stage II was 33.7 μm (SD = ±5.37), for Stage III 49.4 μm (SD = ±1.08), and for Stage IV 49.94 μm (SD = ±0.17). The maximum oocyte diameter observed was 50 μm and corresponded to individuals of at least 50 mm in length. Sizes of mussels at each stage of gonadal development are shown in Table 1. We observe partial spawning in both males and females; all spawned gonads had remnant mature oocytes.

Table 1. Size and estimated age of *Perna viridis* from La Restinga Lagoon, Venezuela at each stage of gonadal development.

Stage	Description of gonad development	Male mean shell length (mm)	Female mean shell length (mm)
0	No follicle development	<18	<18
I	Early development of follicles	20 SD = 1.69	20 SD = 1.86
II	Active development of follicles and gametes	25 SD = 1.02	25.5 SD = 2.37
III	Ripe	27 SD = 0.90	45.6 SD = 1.70
IV	Partially gamete release	>40	>90

DISCUSSION

The onset of sexual maturation of *Perna viridis* in Asia occurs at a shell length of 15–30 mm (Siddall 1980, Vakily 1989). At La Restinga Lagoon, the size at first maturity was included within this range (over 20 mm) and females larger than 25.5 mm exhibited fully grown oocytes. In addition, individuals from other localities having similar temperature ranges to La Restinga Lagoon have shown similar size ranges at first maturity (Cheong, and Chen 1980, Vakily 1989, Agard *et al.* 1992). On the other hand, Walter (1982), who studied the reproduction and growth of *P. viridis* from mussel farms at the tropical locality of Quezon (The Philippines), reported that spawning started at 20 mm in males and 24 mm in females. These results differ from ours because we found that the size of *P. viridis* at spawning was 27 and 45.6 mm for males and females, respectively.

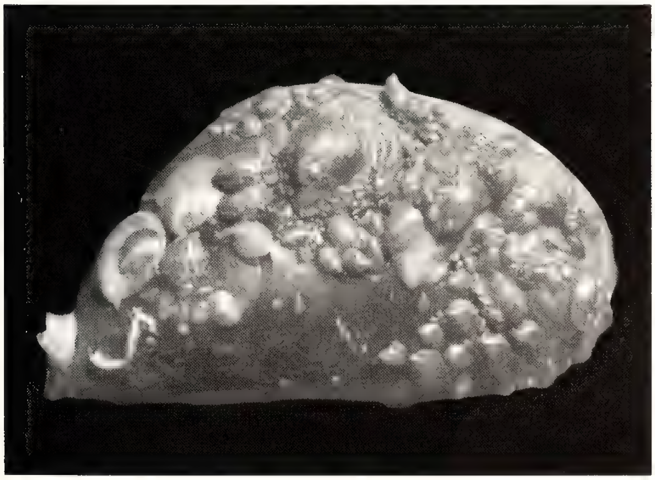

Figure 3. Specimen of *Perna viridis* from La Restinga Lagoon (70 mm shell length) with a large number of epizoic *Crepidula aplysioides*.

Following Ingrao *et al.* (2001), we suggest that males and females of *Perna viridis* started the process of gonadal maturation at an age of approximately 4 months. Complete sexual maturity (stage III) was reached at approximately 4 months in males and 7 months in females. Spawning (stage IV) was reached at an age 5–6 months and 12 months in males and females, respectively.

The salinity range of 16–58 ppt at La Restinga (Segnini de Bravo *et al.* 1998, Scelzo 1999) is considerably wider than the range of salinity tolerance of 31–35 ppt previously reported for this species (Cheong and Chen 1980, Vakily 1989). Mean monthly water temperature at the Lagoon varies annually between 27–30°C (Scelzo 1999) and absolute water temperature can reach up to 40°C (Segnini de Bravo *et al.* 1998). The fact that individuals of *Perna viridis* were able to colonize this extreme environment reveals the species' remarkable opportunistic nature, which is a distinctive feature of invasive species. The absence of the local mussel *Perna perna* at the Lagoon may be due to temperature constrains. Several authors have documented the effect of temperature on *P. viridis:* In the Philippines, gametogenesis was inhibited at temperatures higher than 28°C (Walter 1982); in laboratory conditions, mussels from mainland Venezuela suffered high mortality (over 50%) when exposed to 34.5°C (Segnini de Bravo *et al.* 1998); and in the Gulf of Mexico the maximum water temperature tolerated by this species is 33°C (Hicks *et al.* 2001). The wider range of temperature tolerance for *P. viridis* compared to that for *P. perna* may influence the spatial distribution of both species in the area. *Perna viridis* and *P. perna* do coexist in the oceanic coast adjacent to the Lagoon.

At La Restinga Lagoon, the invasive *Perna viridis* pro-

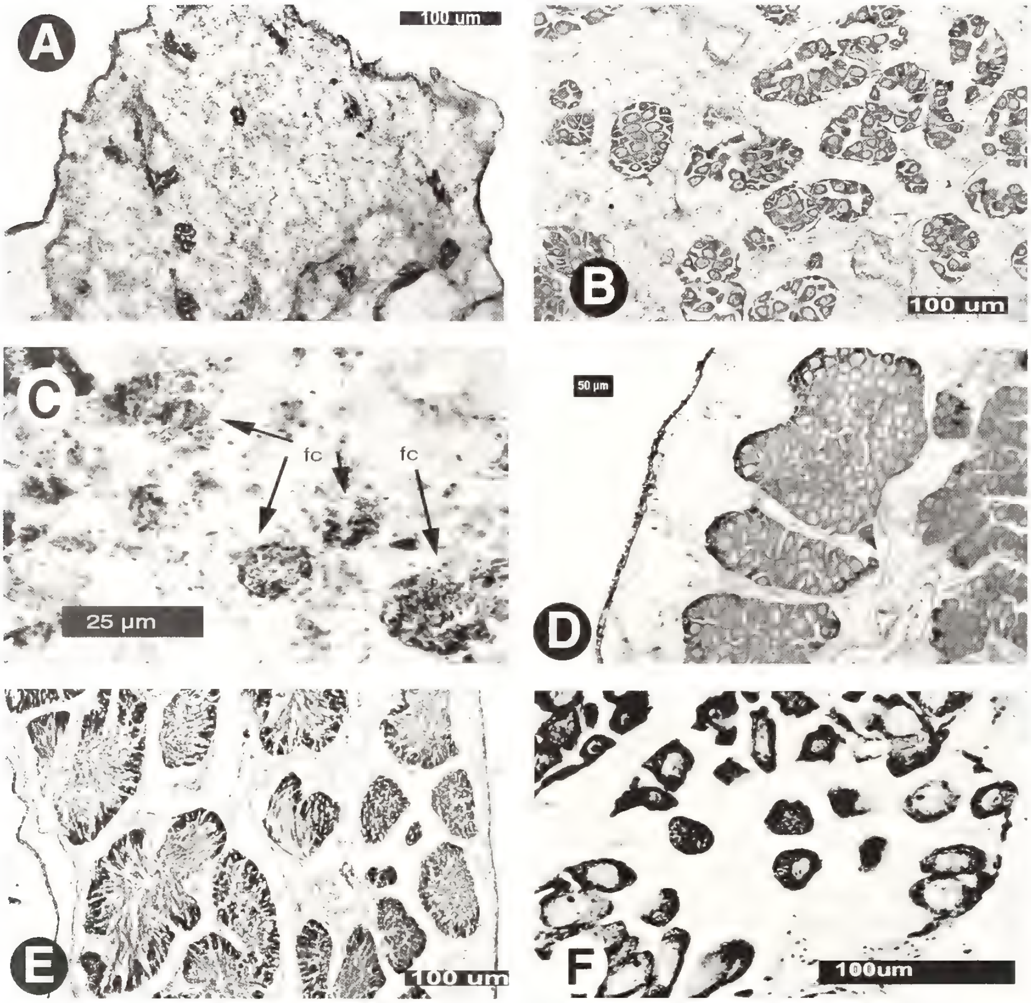

Figure 4. Light micrographs of histological sections of the mantle of *Perna viridis*. A. Stage I. Early follicle development (arrow) in a mussel of 20 mm shell length. Scale bar = 100 µm. B. Stage II. Developing gonad in a female of 25 mm shell length. Scale bar = 100 µm. C. Stage II .Immature male (25 mm shell length). Scale bar = 100 µm; fc: follicles. D. Stage III. Mature gonad in a female of 45 mm shell length. Scale bar = 50 µm. E. Stage IV. Male of 42 mm shell length at spawning. Scale bar = 100 µm F. Stage IV. Follicles in a female of 91 mm shell length after spawning. Scale bar = 100 µm.

vides additional smooth surfaces for the settlement of marine invertebrate larvae, thus leading to a potential change in the composition of the fouling community. An impressive example of this is the abundance of the calyptreid *Crepidula aplysioides*. Before the colonization of La Restinga by *P. viridis,* this relatively sessile gastropod was frequently attached to the lower valves of the oyster *Crassotrea rizophorae,* and now it is attached mainly to the valves of *P. viridis* (Miloslavich and Penchaszadeh 2003). A shift in the attachment substrate has also been reported by Thieltges *et al.* (2003) for *Crepidula fornicata* (Linnaeus, 1758) from oysters to the mussel *Mytilus edulis* Linnaeus, 1758 in the Wadden Sea. Benson *et al.* (2001) mentioned the importance of environmental impacts on marine communities in Florida resulting from the settlement of *P. viridis.* As a result of the reproduction and dispersal of this species within the La Restinga Lagoon, there is an increased availability of hard and smooth substrates for the establishment of fouling invertebrates.

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Growth of *Biomphalaria glabrata* (NMRI strain) and *Helisoma trivolvis* (Colorado strain) under laboratory conditions

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Abstract: Growth of two planorbid snails, *Biomphalaria glabrata* (NMRI strain) and *Helisoma trivolvis* (Colorado strain), was studied from 0 to 16 weeks under controlled laboratory conditions. Cultures of both species were maintained in mason jars containing artificial spring water at 23°C and fed boiled Romaine lettuce leaf *ad libitum*. Growth was studied on an almost weekly basis by determining the shell diameter and blotted wet weight of the snails. The shell diameter and wet weight of *Helisoma trivolvis* was significantly greater than that of *Biomphalaria glabrata* (Student's t-test, $P < 0.05$) from 9 to 16 weeks. Both snails laid viable eggs in culture. The laboratory conditions described herein are satisfactory for maintaining both of these species.

Key words: laboratory maintenance, pulmonates

The pulmonate gastropod *Biomphalaria glabrata* (Say, 1816) serves as a first intermediate host for *Schistosoma mansoni* Sambon, 1907. *Biomphalaria glabrata* and *Helisoma trivolvis* (Say, 1816) (Colorado strain) serve as second intermediate hosts for *Echinostoma caproni* (Richard, 1964) and *Echinostoma trivolvis* (Cort, 1914) (Frazer and Fried 1998, Fried and LaTerra 2002, Fried *et al.* 2002, Fried and Kim 2003). Studies of these host-parasite relations have required a dependable source of juvenile and adult planorbid snails. Incidental to our studies on host-parasite relationships of larval echinostomes and schistosomes in *H. trivolvis* and *B. glabrata*, we have examined growth of these planorbids based on shell diameter and blotted wet weight of juvenile and sexually mature adult snails. The results of our growth study are described herein.

METHODS

Stock cultures of *Biomphalaria glabrata* (NMRI strain), about 5–8 mm in shell diameter, were obtained from Dr. Fred Lewis, Schistosomiasis Laboratory, Biomedical Research Institute (Rockville, Maryland, USA). Stock cultures of *Helisoma trivolvis* (Colorado strain) have been maintained in our laboratory in continuous culture for more than 15 years (see Fried *et al.* 1987). Groups of 20 snails of each species were maintained at 23 ± 1°C in aerated glass containers (mason jars) each containing 800 ml of artificial spring water (ASW) (Ulmer 1970) under diffuse overhead fluorescent light for 12 hr per day. These cultures were fed *ad libitum* on boiled Romaine lettuce leaf and the water was changed twice weekly. To collect snail eggs, strips of plastic, 4 cm × 4 cm, were placed in both the *H. trivolvis* and *B. glabrata* cultures. The strips containing eggs were removed to finger bowl cultures (11-cm diameter with 150 mL ASW) containing 2 cm × 2 cm pieces of lettuce, and the juveniles (50 per bowl) were allowed to grow. Once the juvenile snails reached 4–5 mm in shell diameter, they were moved to mason jars and maintained as described above. Newly hatched snails were measured with a calibrated ocular micrometer. Measurements of shell diameters of 2 to 16 week old *H. trivolvis* and 4 to 16 week old *B. glabrata* were made weekly to the nearest 0.5 mm with a millimeter ruler. Individuals that were 4–16 weeks old of both species were weighed weekly by drying them on a paper towel prior to weighing them on an analytical balance.

RESULTS

The diameters of shells of newly hatched snails of both species ranged from 0.7–1.0 mm. The mean shell diameters of snails of both species were about 4 mm at 5 weeks (Fig. 1A). After 6 weeks, the shell diameter of *Helisoma trivolvis* increased more rapidly than that of *Biomphalaria glabrata.* The shell diameter of *H. trivolvis* was significantly greater than that of *B. glabrata* from 9 to 16 weeks (Student's t-test, $P < 0.05$). By week 16, the shell diameter increased to approximately 10 mm for *B. glabrata* and 12 mm for *H. trivolvis.* Egg laying first occurred when *B. glabrata* reached approximately 10 mm (14 week old) and *H. trivolvis* reached approximately 7 mm (9 week old).

The mean blotted wet weights for snails of both species were approximately 20 mg at week 5 and reached about 50 mg by week 8. Beyond that the growth of *Helisoma trivolvis* was significantly greater (Student's t-test, $P < 0.05$) than

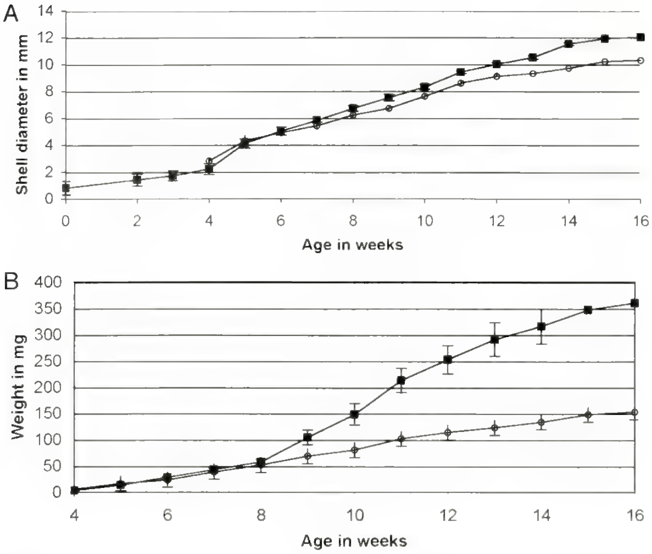

Figure 1. A, Growth (increase in mean shell length) of snails under laboratory conditions. Closed squares represent mean shell diameter ± standard error for *Helisoma trivolvis* (Colorado strain) and open circles represent mean shell diameter ± standard error for *Biomphalaria glabrata* (NMRI strain). Where standard error bars are not seen, they were smaller than the symbol for the mean. B, Growth (increase in mean blotted wet weight) of snails under laboratory conditions. Closed squares represent mean blotted wet weight for *Helisoma trivolvis* (Colorado strain) and open circles represent mean blotted wet weight for *Biomphalaria glabrata* (NMRI strain). Where standard error bars are not seen, they were smaller than the symbol for the mean.

that of *Biomphalaria glabrata*, reaching greater than 350 mg by week 16 compared to about 150 mg for *B. glabrata* at that time (Fig. 1B). Although quantitative data on lettuce consumption by snails in the cultures were not made, at week 8 and beyond, individuals of *B. glabrata* consumed about 100 mg (blotted wet weight) of lettuce snail^{-1} week^{-1}, whereas individuals of *H. trivolvis* consumed about 200 mg snail^{-1} week^{-1}.

DISCUSSION

Many laboratories maintain *Biomphalaria glabrata* at 26–28°C to obtain optimal development of the intramolluscan stages of *Schistosoma mansoni* and also supplement a lettuce diet with high-protein fish food to raise large numbers of snails to reach sexual maturity rapidly and attain maximal shell diameter (Lewis *et al.* 1986). Inadequate diets, *i.e.*, lettuce alone, have resulted in individuals of *B. glabrata* that grew more slowly and took longer to reach patency than cohorts on diets with nutrient supplements (Eveland and Ritchie 1972, Coles 1973).

Information on dietary needs and temperature optima for maintaining the Colorado strain of *Helisoma trivolvis* are not available. This snail is a temperate zone planorbid and a temperature of 23°C appeared adequate for good growth and sexual maturation. *Biomphalaria glabrata* is a tropical and subtropical species and requires temperatures higher than 23°C for optimal growth. Although development of *B. glabrata* was slower under these conditions than reported by other workers (Lewis *et al.* 1986), our snails produced eggs by week 14. These eggs hatched and were capable of developing into sexually mature adults under our laboratory conditions. Therefore, the use of 23°C and a Romaine lettuce diet is adequate for the maintenance of both of these species in the laboratory.

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Distribution of the molluscan fauna in subtidal soft bottoms of the Ensenada de Baiona (NW Spain)

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Abstract: The composition and distribution of the molluscan fauna on the subtidal soft bottoms of the Ensenada de Baiona (Galicia, NW Spain) were studied by means of quantitative sampling and multivariate analyses. Several assemblages were determined whose faunistic and sedimentary compositions mostly coincided with other "communities" or "facies" reported along the Galician and the European coasts, especially with those found in the Ría da Coruña and the Ría de Ares and Betanzos. The faunal distribution seemed to be mainly conditioned by a sedimentary gradient that was defined by a decreasing grain size from the mouth towards the inner margins of the inlet. A facies of the *Venus fasciata* community is present in coarser sandy sediments of the outer inlet area; the communities characterized by *Venus gallina* and *Tellina fabula- Tellina tenuis* were found in the fine sand/muddy sand bottoms at the center and margins of the inlet. The fauna of the muddy sediment on the sheltered side of Baiona Harbor showed similarities with the *Syndosmya* (=*Abra*) *alba* community, possibly due to a substitution of the *Tellina* spp. community. This phenomemon may be related to an increase in the silt/clay content of the sediment, which might have resulted from a change in current dynamics after the construction of the Baiona Harbor jetty, as indicated by previous studies.

Key Words: faunal composition, distribution, assemblages, Galicia

During the last thirty years, a number of important studies have been carried out on the marine benthic fauna of the Iberian Peninsula, both at the ecological and taxonomic levels. There has been an ongoing interest in the so-called "rías" of Galicia (NW Spain), which are a particular kind of estuarine system. The rías had their origin in flooded river valleys and have a high primary productivity due to upwellings and regular incoming of nutrients (Nombela *et al.* 1995). The great economic and social importance of these systems (fisheries, bivalve culture on rafts, shellfish resources) would greatly benefit from a scientific study of the environment, especially of the benthic communities, which have traditionally been considered as good indicators of the conditions of marine bottoms (Bellan 1967, 1984, Pearson and Rosenberg 1978, Gray and Mirza 1979, Bellan *et al.* 1988, Warwick 1988). Compared to other rías, there are few studies of soft bottom benthic communities of the Ría de Vigo, and many of these are restricted to intertidal areas (Viéitez 1976, 1981, Anadón 1980, Viéitez and López-Cotelo 1982, López-Jamar and Cal 1990, Abella *et al.* 1996). Although its malacological fauna is well known from the wealth of taxonomic literature available (MacAndrew 1849, Hidalgo 1886, Figueras 1956, Rolán 1983, Rolán *et al.* 1989, Rolán-Álvarez and Rolán 1989) there is a lack of sinecological studies (Viéitez 1976).

The Ensenada de Baiona is a small inlet located on the southern margin of the Ría de Vigo that is relatively unaltered by human activities. The main objectives of this project were to characterize the composition and distribution of the molluscan fauna on the subtidal soft bottoms of the Ensenada de Baiona and to study the influence of the measured environmental variables on the distribution patterns, thus providing a reference case study for future comparative studies on the ecology of the communities.

MATERIALS AND METHODS

Study area

The Ensenada de Baiona is located on the southern margin of the mouth of the Ría de Vigo, between 42°7′N-42°9′N and 8°51′W-8°49′W (Fig. 1). Its mouth is oriented towards the West, and the dominant winds are W-SW in winter (Alejo *et al.* 1999). It is semicircular in shape, is 2.7 km wide, 3.3 km long, and has a maximum depth of 16 m. Previous studies showed that the sediments from the inlet are of a sandy nature, usually with low values in organic matter, and show a clear decrease in grain size from the outer part towards the inner margins and sheltered areas near Baiona Harbor (Alejo and Vilas 1987, Alejo *et al.* 1999).

Sample collection

A total of 21 stations were sampled to cover the extension of the inlet (Table 1). Quantitative sampling was carried out during December 1995 using a Van Veen grab with a sampling area of 0.056 m^2. Five replicates were taken at each

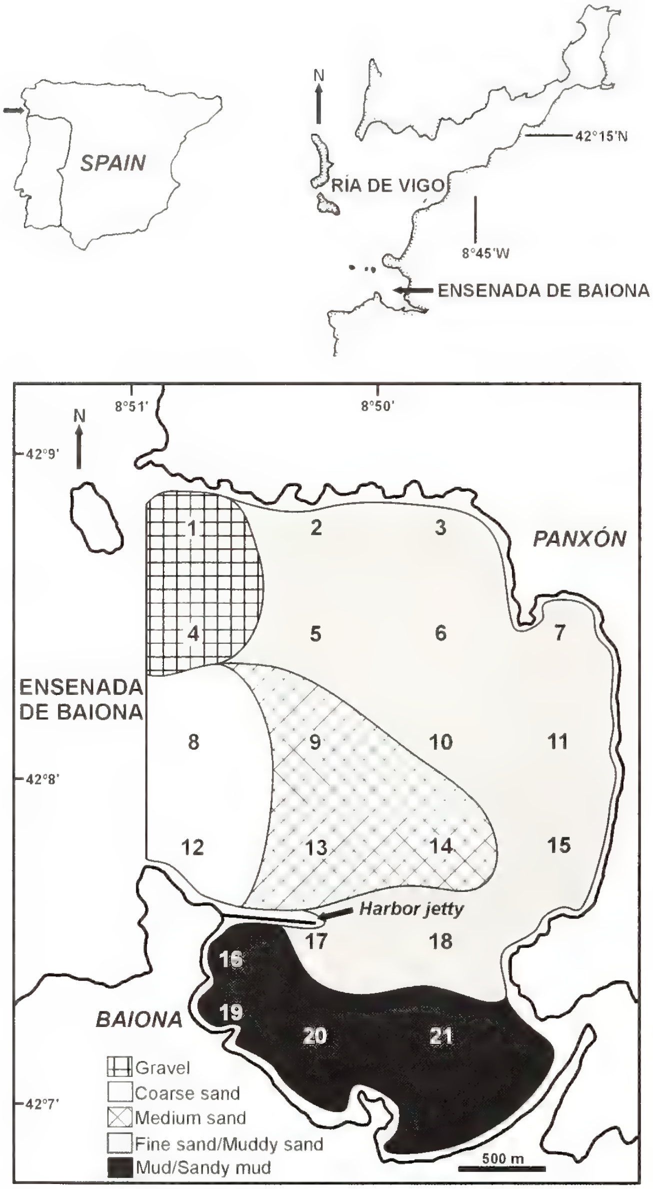

Figure 1. Locations of sampling stations and distribution of sedimentary types in the Ensenada de Baiona.

station, which accounted for a total area of 0.28 m^2. Samples were sieved through a 0.5 mm mesh and fixed in 10% buffered formalin for later sorting and identification of the fauna. An additional sediment sample was taken at each station to analyze the granulometric composition, carbonates, and organic matter contents. The following granulometric fractions were considered: gravel (GR, >2 mm), very coarse sand (VCS, 2-1 mm), coarse sand (CS, 1-0.5 mm), medium sand (MS, 0.5-0.25 mm), fine sand (FS, 0.25-0.125 mm), very fine sand (VFS, 0.125-0.063 mm), and silt/clay (<0.063 mm). Median (Q_{50}) and sort coefficient (S_o) (Trask 1932) were also determined for each sample. Median is the size particle for which 50% of the grains are coarser (McManus 1988). Sort coefficient was considered as $S_o = \sqrt{Q_{25}/Q_{75}}$, where Q_{25} and Q_{75} are the 25th and 75th percentiles. Sedimentary types were characterized according to Junoy (1996). Carbonate content (%) was estimated by treatment of the sample with hydrochloric acid, and the total organic matter content (TOM, %) was estimated from the weight loss after placing samples in a furnace for 4 h at 450°C.

Data analyses

Several univariate measures were calculated for each sampling station: total abundance (N), number of species (S), the Shannon-Wiener diversity index (H′, $\log_2$), and Pielou's evenness (J). Molluscan assemblages were determined through non-parametric multivariate techniques as described by Field *et al.* (1982) using the PRIMER v5.0 (Plymouth Routines in Multivariate Ecological Research) software package (Clarke and Warwick 1994). A similarities matrix between sampling stations was constructed by means of the Bray-Curtis similarity coefficient by first applying fourth root transformation on species abundance to downweight the contribution of the most abundant species. From this matrix, a classification of the stations was performed by cluster analysis based on the group-average sorting algorithm, as well as an ordination by means of non-metrical multidimensional scaling (MDS). Possible differences in faunistic composition between samples were tested using the 1-way ANOSIM test. The SIMPER program was next used to identify species that greatly contributed to differentiate station groups. The species present in each group of stations were further classified according to the Constancy and Fidelity indexes (Glémarec 1964, Cabioch 1968). For any given station group, species with more than 1% of the total specimens were considered as dominants (Junoy and Viéitez 1990).

The possible relationship between molluscan distribution in the inlet and the measured environmental variables were researched using the BIO-ENV procedure (belonging to the PRIMER package) and the canonical correspondence analysis (CCA) using the CANOCO v4.02 (Canonical Community Ordination) package (Ter Braak 1988). The forward selection was employed in the latter to detect which variables explained the most variance in the species data. The following variables were considered in these analyses: TOM (%), carbonates (%), all of the granulometric fractions (%), median (mm), sort coefficient, and depth (m). All variables expressed in percentages were previously transformed by log (x+1). Stations 1 and 4 were discarded because of their stony nature, which made interpretation of the analyses difficult, and because of insufficient sample content for Station 4.

Table 1. Position, depth, and sedimentary characteristics of sampling stations in the Ensenada de Baiona. See Fig. 1 for a map of station locations. Q_{50}, median grain size; S_o, sort coefficient; $CO_3^=$, carbonates; TO, total organic matter.

Station	Position (N)	Position (W)	Depth (m)	Gravel (%)	Sand (%)	Silt/Clay (%)	Q_{50} (mm)	Sedimentary type	S_o	$CO_3^=$ (%)	TOM (%)
1	42°08′50″	08°50′52″	7	94.23	5.74	0.03	5.000	Gravel	Moderate	24.26	1.48
2	42°08′50″	08°50′15″	7	3.52	89.96	6.52	0.135	Muddy sand	Moderate	32.39	1.91
3	42°08′50″	08°49′44″	4	0.01	87.19	12.80	0.088	Muddy sand	Mod. well sorted	29.67	2.27
4	42°08′30″	08°50′52″	12	–	–	–	–	–	–	–	–
5	42°08′30″	08°50′15″	11	0.15	88.41	11.45	0.092	Muddy sand	Mod. well sorted	34.37	1.70
6	42°08′30″	08°49′44″	7	0.09	90.66	9.25	0.093	Muddy sand	Mod. well sorted	37.59	2.10
7	42°08′30″	08°49′13″	3	0.04	96.44	3.53	0.146	Fine sand	Moderate	48.33	2.05
8	42°08′10″	08°50′52″	12	10.29	88.40	1.31	0.831	Coarse sand	Moderate	68.94	1.48
9	42°08′10″	08°50′15″	10	1.24	95.71	3.06	0.350	Medium sand	Moderate	82.67	2.28
10	42°08′10″	08°49′44″	8	5.84	88.94	5.22	0.140	Muddy sand	Moderate	49.13	2.20
11	42°08′10″	08°49′13″	3	0.18	84.28	15.54	0.099	Muddy sand	Mod. well sorted	44.20	2.50
12	42°07′50″	08°50′52″	9	8.27	91.71	0.03	0.900	Coarse sand	Moderate	61.31	1.32
13	42°07′50″	08°50′15″	8	0.44	96.47	3.09	0.337	Medium sand	Moderate	79.68	2.07
14	42°07′50″	08°49′44″	9	1.14	95.70	3.16	0.310	Medium sand	Moderate	80.35	2.18
15	42°07′50″	08°49′13″	4	0.03	95.83	4.14	0.140	Fine sand	Moderate	45.00	2.32
16	42°07′30″	08°50′45″	4	0.04	9.85	90.11	0.017	Mud	Poor	5.81	12.05
17	42°07′30″	08°50′15″	7	9.63	84.18	6.19	0.230	Muddy sand	Moderate	72.91	3.18
18	42°07′30″	08°49′44″	8	2.11	94.08	3.82	0.230	Fine sand	Moderate	75.33	2.48
19	42°07′19″	08°50′45″	2	2.53	22.95	74.52	0.023	Mud	Poor	7.11	8.45
20	42°07′10″	08°50′15″	3	0.17	21.67	78.17	0.022	Mud	Poor	6.85	7.28
21	42°07′10″	08°49′44″	3	0.19	50.61	49.20	0.064	Sandy mud	Poor	4.36	2.82

RESULTS

Sediments

The results of sedimentary analyses were mostly similar to previous works in the area (Alejo and Vilas 1987, Alejo *et al.* 1999). Most of the sampled stations were of a sandy nature (Table 1, Fig. 1), with low percentages in silt/clay and low contents in TOM. Highest carbonate contents were recorded in the outer and central parts of the inlet (61.3-82.7%), and values decreased towards the inner margins. However, four stations located in the sheltered area of the inlet, namely Stn. 16, 19-21, were found to be muddy (Q_{50}: 0.064-0.017 mm), with TOM contents ranging from moderate to high (2.8-12.0%), in contrast to the very fine sand and fine sand bottoms recorded by Alejo *et al.* (1999) from samples that were taken between 1985-1986.

Abundance, species richness and diversity

A total of 9862 individuals belonging to 94 species were collected. Bivalves were the dominant group in terms of abundance (82.3%), followed by gastropods (16.5%) and polyplacophorans (1.2%). Scaphopods were only represented by two specimens (one species). The numbers of gastropod and bivalve species were similar (50 and 40, respectively), while polyplacophorans were represented by 3 species. The bivalves *Mysella bidentata* (Montagu, 1803), *Fabulina fabula* (Gronovius, 1781), and *Goodallia triangularis* (Montagu, 1803) were the most abundant species in the inlet, accounting for more than 50% of total molluscan abundance. The first two were mainly distributed along a number of fine sediments (fine sand to mud) and the latter was distributed in coarser sandy sediments (coarse and medium sand). The only relatively abundant gastropod was the caecid *Caecum imperforatum* (Kanmacher, 1798), which was mainly present in medium sand bottoms.

Values of univariate measures are shown in Table 2. Lowest abundance values were recorded at Stn. 16 (29) and Stn. 1 (78), while the highest were recorded at Stn. 21 (1098) and Stn. 8 (1034), chiefly due to the abundance of *Thyasira flexuosa* (Montagu, 1803) and *Goodallia triangularis*, respectively. Species richness fluctuated between 6 (Stn. 3, 16) and 36 (Stn. 17, 18), while diversity ranged between 1.02 (Stn. 6) and 3.24-3.50 bits (Stn. 17, 18, 1). Evenness showed low values (0.29-0.38) on bottoms with a high dominance of *G. triangularis* (Stn. 8, 12) and where a low specific richness coupled with a high dominance by *Fabulina fabula* was detected (Stn. 5, 6). Species richness showed a positive correlation through non-parametric Spearman's correlation coefficient with gravel ($p < 0.01$), very coarse sand, medium sand, and median ($p < 0.05$) and negative correlations with silt/clay

Table 2. Species richness (S), total abundance (N), Shannon Wiener's diversity index (H′), and Pielou's evenness (J) for each sampling station in the Ensenada de Baiona.

Station	S	N	H′	J
1	19	78	3.50	0.82
2	25	270	3.21	0.69
3	6	329	1.55	0.60
4	29	749	2.69	0.55
5	11	351	1.31	0.38
6	12	352	1.02	0.29
7	11	151	2.16	0.62
8	14	1034	1.45	0.38
9	16	428	2.54	0.63
10	19	174	2.71	0.64
11	9	199	2.25	0.71
12	18	909	1.32	0.32
13	13	209	2.93	0.79
14	27	486	3.10	0.65
15	15	811	2.01	0.52
16	6	29	2.10	0.81
17	36	727	3.24	0.63
18	36	868	3.26	0.63
19	12	346	1.98	0.55
20	11	259	2.61	0.75
21	24	1098	2.87	0.63

($p < 0.05$), while total abundance was positively correlated with coarse sand ($p < 0.05$).

Multivariate analysis

Cluster analysis revealed the presence of two large groups of stations (Fig. 2), those with coarser sediments (Stn. 1, 4, 8, 9, 12-14) and those with finer sediments (Stn. 2, 3, 5-7, 10, 11, 15-21), in which the following assemblages were distinguished: group A (Stn. 1, 4: gravel bottoms); group B, with subgroups B1 (Stn. 8, 12: coarse sand) and B2 (Stn. 9, 13, 14: medium sand); group C, with subgroups C1 (Stn. 2, 10, 17, 18: fine/muddy sand with a significant content of medium sand) and C2 (Stn. 3, 5-7,11, 15: fine/muddy sand with lower content of medium sand); and group D (Stn. 16, 19-21: sandy mud/mud bottoms). MDS ordination (Fig. 3) showed similar results to those of the dendrogram, with an acceptable stress value (0.12). This ordination also indicated an affinity between Stn. 10 (C1) and group C2, and between Stn. 18 (C1) and group B2, as well as showing the proximity of Stn. 8 and 12. The ANOSIM test pointed out the existence of significant differences in the faunistic composition between all stations (global R: 0.916, p: 0.001), the exception being Stn. 8 and 12 (R: 0.024, p: 0.413).

Results of the SIMPER analysis are shown in Table 3. The bivalves *Mysella bidentata* and *Goodallia triangularis* and several epifaunal species, such as *Acmaea virginea* (Müller, 1776), *Gibbula tumida* (Montagu, 1803), and *Leptochiton cancellatus* (Sowerby, 1840), contributed greatly to differentiate gravel bottoms from coarse sand stations, while the presence of *Thracia papyracea* (Poli, 1791), *Retusa umbilicata* (Montagu, 1803), *M. bidentata*, and the different abundance of *Digitaria digitaria* (Linné, 1758) and *G. triangularis* explained most of the dissimilarity between groups B1 and B2. The medium sand assemblage (B2) differed from finer sediments of group C1 due to the presence of *G. triangularis*, *Caecum imperforatum*, and *Philine scabra* (Müller, 1776) in the former, and of *Fabulina fabula*, *Nassarius reticulatus* (Linné, 1758), and *M. bidentata* in the latter. The dissimilarities between the two assemblages of group C were explained by *Angulus tenuis* (da Costa, 1778), *Cuneus vittatus* da Costa, 1778 (both of them with higher abundances in C2), *R. umbilicata*, *Chamelea striatula* (da Costa, 1778), and *T. papyracea* (the last three being more abundant in C1). Differences between groups C1-C2 and the muddy bottoms of group D were mainly due to the presence in the latter of *Thyasira flexuosa*, *Loripes lacteus* (Linné, 1758), and *Bittium reticulatum* (da Costa, 1778).

The BIO-ENV procedure (Table 4) showed that the best combinations of environmental variables through the highest correlations with faunistic data were those composed of carbonates/TOM/coarse sand/median and the first three variables with very fine sand and sort coefficient. Depth was not part of any of the best combinations. The median was the variable with the best value (p_w: 0.715) when each variable was considered alone, followed by very fine sand (p_w: 0.594). On the other hand, the forward selection of CCA selected the median, sort coefficient, and content in medium sand as the variables explaining most of the variance in the species data (p: 0.001), while gravel, very coarse sand, and carbonates were found to be significant only at the 5% level.

The graphic representation of CCA showed an ordination of stations similar to that of MDS (Fig. 4), axes I and II being the most important (Table 5), accumulating 44.6% of the species variance and 53.9% of species-environment variance. Content of coarse sand, very coarse sand, very fine sand, silt/clay, median, and depth showed a strong correlation with axis I. The correlations with the other axes, however, were less significant. Group D appeared clearly separated from the other assemblages due to its different contents in silt/clay, TOM, and sort coefficient, while sandy sediment assemblages appeared distributed from the right to the left of axis I following an increase in content of fine sand/very fine sand and a subsequent decrease in coarser granulometric fractions, with group C1 located at an intermediate position between groups B2 and C2. Station 10 again appeared close to group C2, while Stn. 8 and 12 ap-

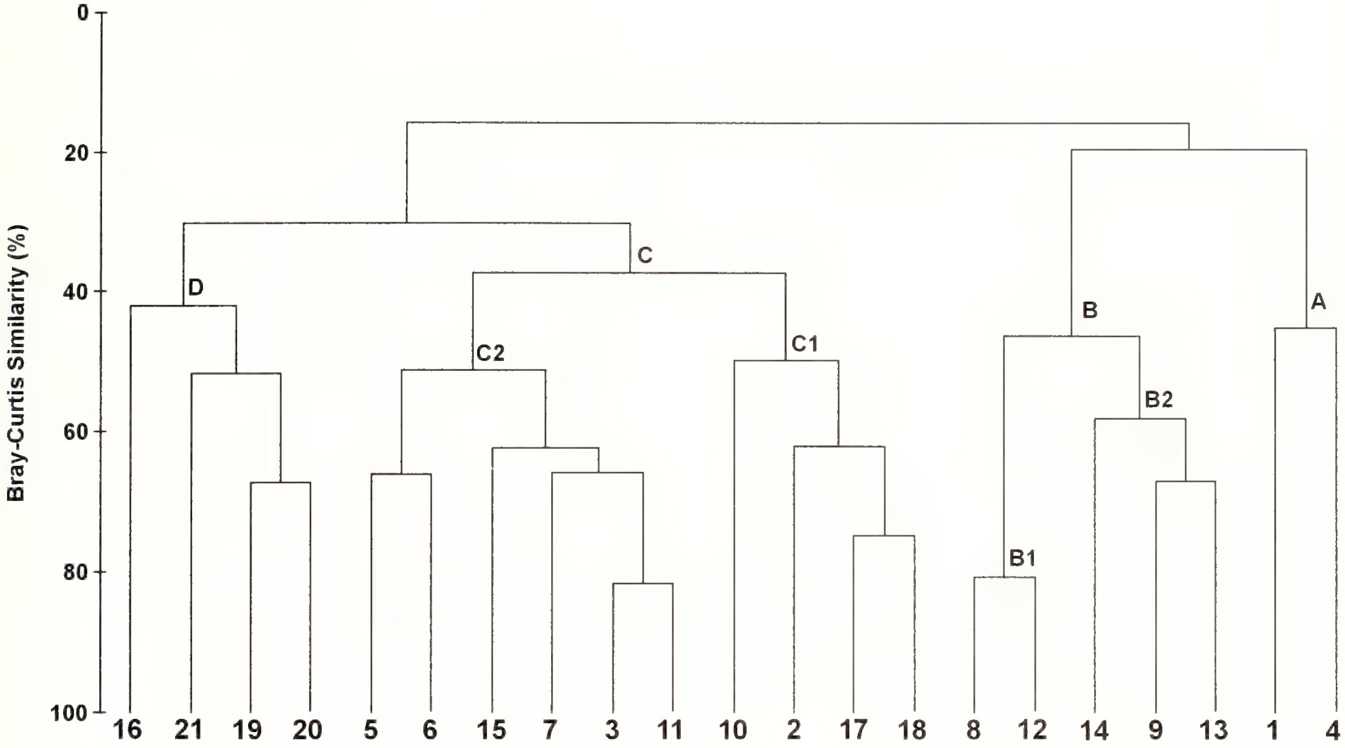

Figure 2. Molluscan assemblages in the Ensenada de Baiona as determined by cluster analysis based on Bray-Curtis similarity coefficient.

peared almost superimposed, which is in consonance with the results obtained by the ANOSIM test. Thus, the different analyses suggested that distribution of fauna in the study area was mainly related to grain size.

Description of assemblages

The dominant species of each assemblage determined by the different multivariate techniques and their Constancy and Fidelity index values are shown in Table 6.

Group A was mainly dominated by epifaunal species, chiefly gastropods and polyplacophorans, although *Mysella bidentata* was the most abundant species at Stn. 4. In spite of their common gravelly nature, Stn. 1 was found to be poorer than Stn. 4 in terms of both species richness and total abundance. This situation could be due to the more heterogeneous nature of the latter, which included valves of *Venerupis rhomboides* (Pennant, 1777) mixed with stones as well as dead rhizoids of *Laminaria* spp., on which were found several individuals of *Helcion pellucidum* (Linné, 1758).

Group B1 was characterized by high abundances of *Goodallia triangularis, Moerella donacina* (Linné, 1758), *Gobraeus tellinella* (Lamarck, 1818), and *Philine scabra*, as well as by the presence of *Retusa mammillata* (Phillipi, 1836) (exclusive), *Clausinella fasciata* (da Costa, 1778) (elective), and *Arcopagia crassa* (Pennant, 1777) (preferential). This assemblage showed high densities (909-1034 ind./0.28 m^2), mainly due to the dominance of *G. triangularis*, which affected diversity (1.32-1.45) and evenness (0.32-0.38) values.

Group B2 included several species shared with group B1, namely *Goodallia triangularis, Moerella donacina, Caecum imperforatum*, and *Digitaria digitaria*, the latter two being more abundant in this assemblage, and shared the presence of *Thracia papyracea, Mysella bidentata*, and *Retusa umbilicata* with group C1. The only constant and exclusive species was the opisthobranch *Philine aperta* (Linné, 1767). The lack of exclusive species and its global faunistic composition indicated transitional bottoms between the coarser sandy stations of the outer inlet and the finer sediments of inner areas. A total of 34 species were recorded, compared to group B1 (20). Most species were represented by fewer than 10 individuals. Group B2 also had a higher diversity (2.54-3.10) and evenness (0.63-0.79) than did B1.

Group C1 was characterized by having the highest total number of species (52), which ranged from 19 to 36 for all stations. Higher specific richness and abundance values were recorded at Stn. 17-18, which showed a greater content of medium sand in spite of a predominance of the fine sand fraction in the assemblage. Diversity ranged from 2.71 to 3.26 and evenness from 0.63 to 0.69. The characteristic species in terms of abundance were *Mysella bidentata, Fabulina*

Table 3. Results of SIMPER analysis. Species were ranked according to their average contributions to dissimilarity between assemblages in the Ensenada de Baiona. Average abundance, ratio value (Dissimilarity/Standard deviation, Diss./SD), and percentage of cumulative dissimilarity were also included.

	Av.Abund.	Av.Abund.	Av.Diss.	Diss./SD	Cum.%
Groups A-B1 (average dissimil.: 81.08)	*Group A*	*Group B1*			
Goodallia triangularis (Montagu, 1803)	1.50	728.50	5.02	3.11	6.19
Mysella bidentata (Montagu, 1803)	203.00	–	4.72	3.43	12.01
Acmaea virginea (Müller, 1776)	50.00	–	4.30	13.83	17.31
Gibbula tumida (Montagu, 1803)	23.00	–	4.15	2.98	22.43
Moerella donacina (Linné, 1758)	1.00	131.00	3.53	3.10	26.78
Leptochiton cancellatus (Sowerby, 1840)	10.50	–	3.10	6.20	30.61
Groups B1-B2 (average dissimil.: 53.87)	*Group B1*	*Group B2*			
Thracia papyracea (Poli, 1791)	–	51.33	4.69	5.55	8.70
Retusa umbilicata (Montagu, 1803)	–	25.33	4.21	4.08	16.52
Mysella bidentata (Montagu, 1803)	–	16.33	3.34	4.12	22.71
Digitaria digitaria (Linné, 1758)	6.50	65.00	2.81	5.59	27.93
Goodallia triangularis (Montagu, 1803)	728.50	58.67	2.66	3.38	32.86
Groups B2-C1 (average dissimil.: 68.65)	*Group B2*	*Group C1*			
Caecum imperforatum (Kanmacher, 1798)	103.00	2.75	3.69	2.79	5.37
Goodallia triangularis (Montagu, 1803)	58.67	–	3.67	3.33	10.72
Fabulina fabula (Gronovius, 1781)	0.33	82.75	3.65	2.95	16.04
Digitaria digitaria (Linné, 1758)	65.00	0.25	3.51	4.39	21.16
Philine scabra (Müller, 1776)	13.67	–	2.59	3.26	24.93
Nassarius reticulatus (Linné, 1758)	0.67	26.25	2.49	2.47	28.56
Mysella bidentata (Montagu, 1803)	16.33	193.00	2.14	2.39	31.68
Groups B2-C2 (average dissimil.: 80.56)	*Group B2*	*Group C2*			
Fabulina fabula (Gronovius, 1781)	0.33	184.83	6.88	3.34	8.54
Caecum inperforatum (Kanmacher, 1798)	103.00	–	6.48	6.27	16.58
Digitaria digitaria (Linné, 1758)	65.00	–	5.53	9.33	23.45
Goodallia triangularis (Montagu, 1803)	58.67	–	5.44	3.27	30.19
Groups C1-C2 (average dissimil.: 61.46)	*Group C1*	*Group C2*			
Angulus tenuis (da Costa, 1778)	1.25	98.33	3.14	2.24	5.11
Cuneus vittatus (da Costa, 1778)	1.00	21.00	3.01	2.15	10.01
Retusa umbilicata (Montagu, 1803)	26.00	0.17	2.62	1.63	14.28
Chamelea striatula (da Costa, 1778)	29.25	1.17	2.58	2.24	18.47
Thracia papyracea (Poli, 1791)	46.75	3.00	2.56	1.42	22.64
Lepidochitona cinerea (Linné, 1767)	7.25	0.17	2.21	2.02	26.23
Moerella donacina (Linné, 1758)	3.75	–	2.02	4.03	29.52
Retusa truncatula (Bruguière, 1792)	2.50	0.33	1.73	2.03	32.33
Groups C1-D (average dissimil.: 69.92)	*Group C1*	*Group D*			
Thyasira flexuosa (Montague, 1803)	–	152.50	4.73	4.72	6.76
Loripes lacteus (Linné, 1758)	–	26.50	3.67	2.19	12.01
Bittium reticulatum (da Costa, 1778)	0.50	62.75	3.42	2.38	16.91
Chamelea striatula (da Costa, 1778)	29.25	0.50	2.98	3.05	21.17
Thracia papyracea (Poli, 1791)	46.75	3.50	2.57	1.42	24.85
Retusa umbilicata (Montagu, 1803)	26.00	0.75	2.46	1.54	28.36
Lepidochitona cinerea (Linné, 1767)	7.25	–	2.36	2.77	31.74
Groups C2-D (average dissimil.: 66.84)	*Group C2*	*Group D*			
Thyasira flexuosa (Montagu, 1803)	–	152.50	7.33	5.23	10.96
Bittium reticulatum (da Costa, 1778)	–	62.75	6.14	3.23	20.15
Loripes lacteus (Linné, 1758)	–	26.50	5.80	2.07	28.83
Angulus tenuis (da Costa, 1778)	98.33	10.00	4.80	1.43	36.02

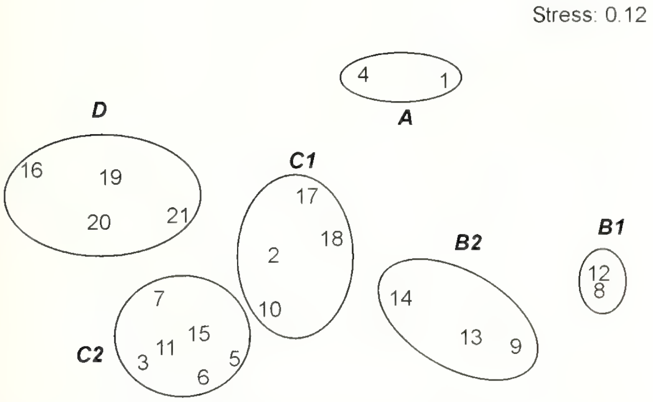

Figure 3. Non-metric multidimensional scaling (MDS) ordination of molluscan assemblages in the Ensenada de Baiona.

fabula, Thracia papyracea, and *Chamelea striatula,* the first two also being found with a remarkable numerical presence in group C2, while *Turritella communis* Risso, 1826 (exclusive), *Mangelia nebula* (Montagu, 1803), *Spisula subtruncata* (da Costa, 1778), and *Venerupis senegalensis* (Gmelin, 1791) (all elective) showed a higher fidelity for this assemblage. On the other hand, the qualitative presence of dead shells of some bivalves (*Lutraria* sp., *Venerupis* spp.) at Stn. 17-18 would explain the appearance of some epifaunal species, namely *Lepidochitona cinerea* (Linné, 1767), *Gibbula cineraria* (Linné, 1758), *G. magus* (Linné, 1758), and *Calyptraea chinensis* (Linné, 1758). In addition, Stn. 17 and 18 also included some typical group B species, such as *Caecum imperforatum, Moerella donacina,* and *Gobraeus tellinella,* but in lower numbers.

Group C2 was remarkably poorer than C1 both in terms of species richness per station (6-15) as well as in total number of species (27), and had a greater percentage of very fine sand. More than 75% of the total number of individuals were *Fabulina fabula* and *Angulus tenuis,* the latter being present in shallower stations (Stn. 3, 7, 11, 15), while the former also reached a high abundance at the deepest stations (Stn. 5, 6). In agreement with these dominances, diversity and evenness showed low values (H': 1.02-2.25; J: 0.29-0.71). The only characteristic species in terms of fidelity were *Pharus legumen* (Linné, 1758) (elective) and *Cuneus vittatus* (preferential).

Group D was comprised of four muddy stations similar to group C2 in species richness (range: 6-24; total number of species: 28), Stn. 16 being the poorest station of all the assemblages from a faunistic point of view (S: 6; N: 29) and had a higher percentage of silt/clay (90%) and TOM (12%). The dominant species in this assemblage as a whole were *Thyasira flexuosa, Mysella bidentata, Bittium reticulatum,* and *Loripes lacteus,* while *Fabulina fabula, Abra alba* (Wood, 1802), and *Angulus tenuis* were also present in substantial numbers in Stn. 21 (sandy mud). The faunistic composition of the last station indicated a transition between the fine sand fauna (*F. fabula, A. tenuis*) and the fauna preferring muddier bottoms (*T. flexuosa, L. lacteus, A. alba*). The following species also showed a high fidelity for this assemblage: *Abra nitida* (Müller, 1776), *Chrysallida fenestrata* (Jeffreys, 1848) (exclusive), and *B. reticulatum* (elective). On the other hand, diversity values ranged between 1.98 and 2.87 and evenness from 0.55 to 0.81.

DISCUSSION

According to our analyses, the distribution of the molluscan fauna in the Ensenada de Baiona seemed to be primarily determined by the grain size gradient, which was characterized by an increase in finer sandy fractions from the mouth of the inlet towards the inner margins. According to Alejo *et al.* (1999), this gradient is a direct consequence of the patterns of wave propagation into the area. On the other hand, depth was not selected as an important factor, due to the short bathymetric range of the inlet. These results are similar to those of Olabarría *et al.* (1998) for subtidal bottoms in Ensenada do Baño (Ría de Ferrol). They recognized sedimentary gradient and organic matter content as the most important factors in faunal distribution. Bergen *et al.* (2001) state that in studies carried out in a relatively narrow depth range the sedimentary differences appear to be more important than the effect of depth.

Table 4. Best combinations of variables obtained through BIO-ENV analysis according to the values of the Spearman's rank correlation (p_w) for the Ensenada de Baiona. $CO_3^=$, carbonates; CS, coarse sand; MS, medium sand; Q_{50}, median; S_o, sort coefficient; TOM, total organic matter; VCS, very coarse sand; VFS, very fine sand.

Number of variables	Correlation (p_w)	Best variable combination
3	0.767	$CO_3^=$–TOM–CS
4	0.788	$CO_3^=$–TOM–CS–Q_{50}
	0.771	$CO_3^=$–CS–VFS–Q_{50}
5	0.789	$CO_3^=$–TOM–CS–VFS–S_o
	0.772	TOM–CS–MS–VFS–S_o
	0.771	CO_3^-–CS–MS–VFS–S_o
6	0.786	$CO_3^=$–TOM–CS–MS–VFS–S_o
	0.785	$CO_3^=$–TOM–CS–VFS–Q_{50}—S_o
	0.774	CO_3^-–TOM–VCS–MS–VFS–S_o
7	0.783	CO_3^-–TOM–CS–MS–VFS–Q_{50}–S_o
	0.771	$CO_3^=$–TOM–VCS–MS–VFS–Q_{50}–S_o

All 12 variables: 0.596

Table 5. Summary of canonical correspondence analysis (CCA) for the Ensenada de Baiona.

Axes	I	II	III	IV		Total inertia
Eigenvalues	0.746	0.456	0.319	0.200		2.694
Species-environment correlations	0.988	0.994	0.973	0.927		
Cumulative percentage variance						
of species data	27.7	44.6	56.4	63.9		
of species-environment relation	33.4	53.9	68.2	77.1		
Sum of all unconstrained eigenvalues					2.694	
Sum of all canonical eigenvalues					2.230	

The molluscan assemblages in the Ensenada de Baiona determined by the different multivariate approaches could be defined using the classic terms of "community," "facies," or "biocoenoses" (Petersen 1918, Thorson 1957, Pères and Picard 1964) by taking into account their sedimentary and faunistic composition. However, the above terms have long been subject to debate, in opposition to the "continuum" concept. The existence of a continuum implies that species are independently distributed along environmental gradients (Curtis 1955). Co-existence or overlap between them in any given assemblage is a function of their responses to these gradients (Mackie *et al.* 1995). In spite of these considerations, classic terms are retained here because of their simplicity and utility as a descriptive method for common reference among scientists (López-Jamar 1981, Junoy 1996, Karakassis and Eleftheriou 1997).

The group B1 had a fauna that could be included among the different varieties of the *Venus fasciata* community of Thorson (1957) or the coarse sands of *Echynocyamus pusillus-Tellina pygmaea* of Glémarec (1973). Several authors have reported the presence of similar assemblages in other areas of Galicia (Ría da Coruña, López-Jamar and Mejuto 1985; Ría de Ares and Betanzos, Troncoso *et al.* 1993), mainly in clean coarse sediments with high contents of biogenic carbonates located at the mouths of the rías, where the hydrodynamism is higher and does not allow the deposition of finer particles (Nombela *et al.* 1987). Although the studied area shared the presence of a number of typical species present in these bottoms (*Arcopagia crassa, Clausinella fasciata, Gobraeus tellinella*), the high dominance of *Goodallia triangularis* would define the presence of a facies of this community in the Ensenada de Baiona. Group B2, which included the medium sand stations, indicated a transition between this *Goodallia* facies and the finer sediments of inner areas of the inlet. This transition was indicated by the presence of species common to both kinds of bottoms, as was also observed by Troncoso and Urgorri (1993) for similar sediments in the Ría de Ares and Betanzos. In the Ensenada de Baiona, however, these bottoms were characterized by a higher dominance of *Caecum imperforatum* and *Digitaria digitaria*.

The communities associated with *Venus gallina* (Thorson 1957) and the boreo-lusitanian *Tellina* (Stephen 1930) would be located respectively in groups C1 and C2, corresponding to the fine sand bottoms of the center and NE margins of the inlet. The *Venus* community gave place to the *Tellina* community by a decrease in the abundance of *Chamelea striatula* and *Thracia papyracea* and by an increase in the numbers of *Cuneus vittatus* and *Angulus tenuis.* However, *Fabulina fabula* showed a high dominance in both assemblages. Thorson (1957) pointed out that a number of varieties of the *Venus gallina* community could appear with a different faunistic composition in function of grain size, wherein the presence of *F. fabula* was favored by finer compacted sands (Mackie *et al.* 1995). Glémarec (1973) defined a *Tellina fabula-Spisula subtruncata* facies that corresponded to the fine sands of *Venus gallina-Mactra corallina* when the amount of silt/clay is usually low. This facies is replaced by a *Pharus legumen-Ophiura texturata* facies when the percentage of fines increases (>5%). However, in our case the highest densities of *F. fabula* were recorded on bottoms with a moderate silt/clay content (9-11%), namely Stn. 5 and 6, while *S. subtruncata* was primarily found at stations belonging to the *Venus gallina* community.

López-Jamar and Mejuto (1985) described a *Tellina* community from Ría da Coruña present through most of its length, mainly in fine sand bottoms (Q_{50} <0.17 mm), with an important dominance of *Fabulina fabula.* According to López-Jamar and Mejuto (1985), this ría is both smaller and shallower than most of the other rías, and is composed of sandy sediments with low organic content that are highly influenced by a strong hydrodynamism, and where muddy sediments are confined to harbor areas. The overall situation can be defined as showing a clear parallelism with the Ensenada de Baiona.

The composition of the muddy bottoms assemblage (group D) had strong affinities with the *Syndosmia* (=*Abra*) *alba* community of Petersen (1918). This community and its several facies and varieties are spread along European coasts in different types of muddy bottoms (Glémarec 1964, Lastra *et al.* 1990, Carpentier *et al.* 1997, Thiébaut *et al.* 1997) as well as in Galician rías (Cadée 1968, Olabarría *et al.* 1998,

Table 6. Median dominance (%), Constancy, and Fidelity values for dominant species in each assemblage of the Ensenada de Baiona. Acc, accessory; Com, Common; Con, constant; Ele, elective; Exc, exclusive; Occ, occasional; Pre, preferential; VC, very common.

	A	B1	B2	C1	C2	D
Species						
Callochiton achatinus (Montagu, 1803)	2.02 (Con/Acc)					
Lepidochitona cinerea (Linné, 1767)	4.86 (Con/Occ)			1.88 (Con/Occ)		
Leptochiton cancellatus (Sowerby, 1840)	3.70 (Con/Exc)					
Acmaea virginea (Müller, 1776)	12.99 (Con/Ele)					
Bittium reticulatum (da Costa, 1778)						15.03 (Con/Ele)
Caecum imperforatum (Kanmacher, 1798)		3.48 (Con/Occ)	27.80 (Con/Occ)			
Calyptraea chinensis (Linné, 1758)	1.80 (Com/Acc)					
Gibbula cineraria (Linné, 1758)	1.74 (Com/Acc)					
Gibbula tumida (Montagu, 1803)	15.13 (Con/Ele)					
Helcion pellucidum (Linné, 1758)	0.93 (Com/Exc)					
Nassarius reticulatus (Linné, 1758)				5.56 (Con/Occ)	2.69 (Con/Occ)	
Philine scabra (Müller, 1776)		1.27 (Con/Acc)	3.89 (Con/Acc)			
Retusa truncatula (Bruguière, 1792)						1.50 (Com/Occ)
Retusa umbilicata (Montagu, 1803)			8.03 (Con/Acc)	5.47 (VC/Acc)		
Abra alba (Wood, 1802)				1.96 (VC/Acc)		4.64 (VC/Acc)
Abra nitida (Müller, 1776)						1.01 (Com/Exc)
Angulus tenuis (da Costa, 1778)					23.91 (VC/Occ)	1.66 (Com/Occ)
Chamelea striatula (da Costa, 1778)				5.60 (Con/Acc)		
Clausinella fasciata (da Costa, 1778)		2.92 (Con/Ele)				
Cuneus vittatus da Costa, 1778					7.07 (Con/Pre)	
Digitaria digitaria (Linné, 1758)			15.86 (Con/Acc)			
Dosinia exoleta (Linné, 1758)	8.69 (Con/Occ)			0.79 (Com/Occ)		
Fabulina fabula (Gronovius, 1781)				20.11 (Con/Acc)	51.28 (Con/Occ)	9.32 (Con/Occ)
Gobraeus tellinella (Lamarck, 1818)		1.07 (Con/Acc)				
Goodallia triangularis (Montagu, 1803)	1.92 (Com/Occ)	75.09 (Con/Acc)	15.89 (Con/Acc)			
Loripes lacteus (Linné, 1758)						13.82 (Con/Exc)
Moerella donacina (Linné, 1758)		13.31 (Con/Occ)	5.71 (Con/Occ)			
Mysella bidentata (Montagu, 1803)	29.40 (Con/Occ)		4.10 (Con/Occ)	34.41 (Con/Occ)	11.01 (Con/Occ)	20.63 (Con/Occ)

Table 6. (continued)

	A	B1	B2	C1	C2	D
Species						
Parvicardium nodosum (Turton, 1822)				1.17 (VC/Occ)		
Pododesmus aculeata (Müller, 1776)	2.47 (Com/Exc)					
Thracia papyracea (Poli, 1791)			13.39 (Com/Acc)	9.64 (VC/Occ)		
Thyasira flexuosa (Montagu, 1803)						29.79 (Con/Exc)
Venerupis senegalensis (Gmelin, 1791)				0.75 (VC/Ele)		

Sánchez-Mata and Mora 1999). In the Ensenada de Baiona an important presence of *Thyasira flexuosa* has been detected, reaching densities of 350 ind/0.28 m^2. López-Jamar and Parra (1997) studied the distribution of this species on the Galician coasts and noted its abundance in muddy sediments with a high organic content, particularly in the Ría da Coruña where densities reached 22,000 ind/m^2. This bivalve has been observed to behave as an opportunist during situations of perturbance (López-Jamar and Mejuto 1988, López-Jamar and Parra 1997). On the other hand, López-Jamar and Mejuto (1985) pointed out that the presence of muddy sediments in the Ría da Coruña, which is mostly sandy, could have been due to the construction of harbor facilities, which would have altered the current dynamics. This situation, in addition to human activities such as organic sewage, could result in a progressive increase of finer granulometric fractions around these areas, and in the case of the Ría da Coruña, result in a replacement of an original *Tellina* community by species with a greater preference for muddier sediments, such as *T. flexuosa* and other oportunistic organisms. We speculate that a similar phenomenon has also occurred in the Ensenada de Baiona, especially if the sediment composition in Baiona Harbor and the surrounding areas are compared with those found during 1985-1986 by Alejo and Vilas (1987). Alejo and Vilas (1987) also noticed that the construction of the harbor jetty during the 1970s could cause a change in currents in following years and a subsequent deposition of fine elements in this area, which our results seem to confirm.

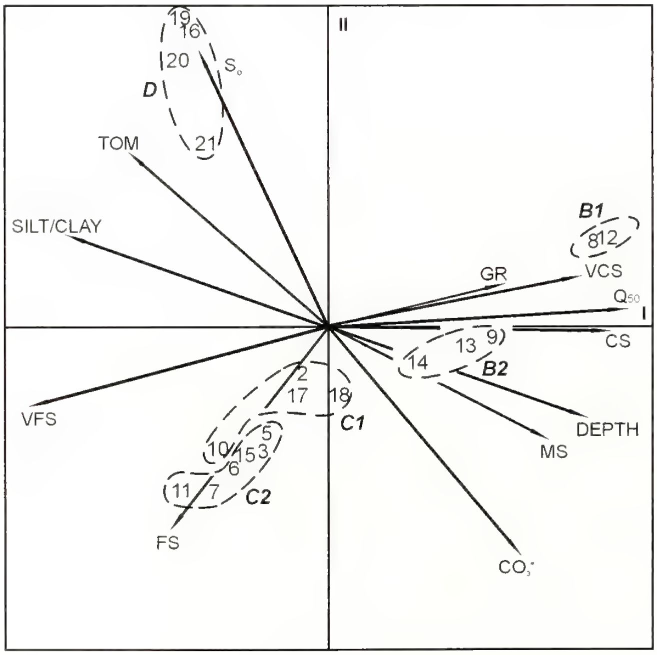

Figure 4. Canonical correspondence analysis (CCA) ordination of stations and environmental variables relative to axes I and II for the Ensenada de Baiona.

On the other hand, phenomena of transition between the *Venus gallina* community and the *Tellina, Amphiura* spp., and *Syndosmia* communities have been reported by different authors for the Galician rías (López-Jamar 1981, Troncoso *et al.* 1993, Sánchez-Mata and Mora 1999), wherein they highlight the difficulty of clearly distinguishing the limits between communities or assemblages. Even though a number of communities or facies have been identified in the study area, we believe there is a continuum in the distribution of molluscs in the Ensenada de Baiona, based on the sedimentary and faunistic compositions of assemblages and the distribution of dominant species, such as *Mysella bidentata, Fabulina fabula, Goodallia triangularis, Thracia papyracea, Chamelea striatula,* and *Angulus tenuis.* These species coexist in all assemblages; differences in their abundances probably reflect their ecological preferences and the environmental conditions found in the inlet.

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Non-marine alien molluscs: The future is a foreign ecosystem*

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The impacts of alien species, along with habitat destruction, are the two most important factors in the global loss of biodiversity. Non-marine molluscs are now transported both deliberately and accidentally all over the world, through human activities (Robinson 1999, Cowie and Robinson 2003). They have numerous impacts, on agriculture (Barker 2002), human health (Rollinson and Chappell 2002), commerce (*e.g.*, Baker 1986, 1996), and the environment, including direct and indirect effects on biodiversity (*e.g.*, Hadfield *et al.* 1993, Cowie 2001). Perhaps the most widely publicized case has been the introduction of zebra mussels, *Dreissena polymorpha* (Pallas, 1771), to North America (*e.g.*, Nalepa *et al.* 2001, Lydeard *et al.* 2004).

Because of the increasing transport of invasive alien species around the world and the increasing public and scientific awareness of the problems they cause, a symposium devoted to invasive alien molluscs, and specifically to non-marine species, seemed timely. The following papers are derived from presentations made at the symposium.

The invited keynote address was given by Geerat Vermeij, who presented a paper asking why certain kinds of snails (for instance the giant African snail *Achatina fulica* Bowdich, 1822) that have invaded islands very successfully have not evolved on islands. This paper, not a definitive review, was intended as a short, speculative, and provocative article, posing more questions than it is possible to provide answers to, and with the hope of stimulating further research.

Other papers addressed a range of topics that included the invasion of North America by zebra mussels (papers by Britton and McMahon and by Padilla), the invasion of South America by the golden mussel, *Limnoperna fortunei* (Dunker, 1857) (Darrigran and Damborenea), a molecular analysis of Asian clams (*Corbicula* spp.) in the New World (Lee, Siripattrawan, Ituarte, and Ó Foighil), new records of alien land snails in North America (Robinson and Slapcinsky), the United States Department of Agriculture's strategy to prevent the importation of *Achatina fulica* and other achatinids into the USA (Smith), and a review of the origins, distribution, impacts, and management of alien non-marine molluscs in the islands of the Pacific (Cowie). In addition to these published papers three presentations were given during the symposium but could not be published as part of these proceedings; these were:

Mark F. Dybdahl (Washington State University, Pullman, Washington): "Invasion success of New Zealand *Potamopyrgus:* Genetic and life-history variation in a clonal invader."

S. J. Nichols (USGS Great Lakes Science Center, Ann Arbor, Michigan): "Alteration of freshwater ecosystems by invasive bivalves, zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena bugensis*)."

Amy R. Wethington (University of Alabama, Tuscaloosa, Alabama; now at Purdue University, Indiana): "Tracking the evolutionary legacy of the biologically invasive, consmopolitan species *Physa acuta*."

I thank all the contributors for making the symposium a success. I have enjoyed working with them not only in organizing the symposium but also in putting together the papers for this issue of the *American Malacological Bulletin*. I especially thank Diarmaid Ó Foighil for inviting me to organize the symposium and for participating extensively in making it happen, including obtaining funding to support it and dealing directly with participants and their travel arrangements. And I thank Janice Voltzow and Ángel Valdés, Editor-in-Chief and Managing Editor, respectively, of the *American Malacological Bulletin*, for being so helpful and supportive throughout the process of preparing the manuscripts for publication. All the papers published herein were formally reviewed by at least two reviewers, whom I thank for their suggestions and guidance.

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Recent introductions of alien land snails into North America*

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Abstract: The number of alien species of terrestrial molluscs in North America continues to increase despite existing quarantine barriers. The effects of non-indigenous species on agriculture, horticulture, or the environment have yet to be ascertained, but are potentially serious. We report the recent introduction of seven species of terrestrial snails into North America. Three Eurasian species, *Monacha cartusiana, Monacha syriaca,* and *Xerolenta obvia* appear to have arrived on shipping containers. The other four species, *Beckianum beckianum, Paropeas achatinaceum, Bulimulus tenuissimus puellaris,* and *Ovachlamys fulgens,* were introduced on plant materials most likely from South and Central America.

Key words: alien species, Gastropoda, pests, agriculture, horticulture

As a consequence of ever-expanding world trade, the opportunity for the introduction of new alien species of molluscs into North America continues to increase (Robinson 1999, Cowie and Robinson 2003). Already more than 80 alien gastropod species have established self-sustaining populations in the United States and Canada (D. G. Robinson, pers. obs.); this number does not include those introduced to the Hawaiian Islands and Puerto Rico, which would make this figure considerably higher. Many of these introductions may pose serious threats to the agriculture and horticulture of the United States, as well as to the natural environment. The costs to the country, both in terms of agricultural or environmental damage, and of control or mitigation efforts, are potentially immense (Pimentel *et al.* 2000). Despite the possible risks posed by alien snails there are few specialists available to identify new introductions or to survey previously reported populations of introduced snails.

Because of the potential for impressive diversity (Emberton 2002) and high levels of local endemicity (Solem 1988) of terrestrial molluscs, malacologists are frequently unfamiliar with the malacofauna of other parts of the world, and a new introduction may remain undetected or unreported for years. For example, the appearance of an unfamiliar snail may result in its description as a species new to science. The malacological literature is filled with cases of widely dispersed alien snails or slugs earning a variety of new names as they are introduced into other parts of the world. Examples include the tiny snail *Paralaoma servilis* (Shuttleworth, 1852) or the ubiquitous slug *Deroceras laeve* (Müller, 1774). Alternatively, unfamiliar snails are often misidentified, sometimes several times, leading to confusion that might slow or hinder mitigation efforts. For example, several hygromiid taxa have been reported from Virginia and North Carolina over the last half century. These include reports of *Helicella caperata* (Montagu, 1846) in Virginia by Wood (1951), a synonym of *Candidula intersecta* (Poiret, 1801) that is also reported as *Jacosta intersecta* (Poiret, 1801) in North Carolina (Dundee 1951). *Helicella striata* (Müller, 1774) was reported from the same area by Hubricht (1953). Afterwards, Beetle (1967) reported the establishment of *Helicella variabilis* (Draparnaud, 1801), a junior synonym of *Cernuella virgata* (Da Costa, 1778), in North Carolina; the latter taxon was also listed by Abbott (1989). It has only relatively recently become clear that all refer to *Cernuella cisalpina* (Rossmässler, 1837). Costly mitigation procedures are now in place to prevent the further spread of this potentially serious agricultural pest. Mitigation costs can be substantially reduced if alien species are detected quickly and eradication efforts are implemented rapidly.

The USDA, through the Cooperative Agricultural Pest Survey (CAPS) program, is currently putting in place mechanisms, including surveys, to detect newly introduced snail and slug species and to delineate better the distributions of previously reported alien species in North America. In this paper we report the recent introduction of a number of synanthropic snails new to North America: two species of Subulinidae, one species of Bulimulidae, one species of Helicarionidae, and three species of Hygromiidae. We present information on their known distributions in North America and provide voucher information for specimens housed at the Florida Museum of Natural History, Gainesville (UF), and the National USDA Mollusk Collection (USDA), currently housed at the Academy of Natural Sciences in Philadelphia.

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FAMILY SUBULINIDAE

Beckianum beckianum (Pfeiffer, 1846)

Material examined

USA, Florida, Broward County: Coral Springs, junction of NW 120th Avenue and NW 41st Street, 26°16.9′N, 80°17.2′W, J. Slapcinsky, 21 September 1999 (UF 278471); Coral Springs, SW of junction of West Commercial Boulevard and NW 64th Avenue, 26°11.6′N, 80°14.4′W, J. Slapcinsky, 17 March 2002 (UF 299924); Coral Springs, Orchid Park, 26°14.7′N, 80°16.2′W, J. Slapcinsky, 8 December 2002 (UF 301063); Davie, 1 km W of Flamingo Gardens, 26°04.5′N, 80°19.4′W, J. Slapcinsky, 8 December 2002 (UF 301296), J. Slapcinsky and D. G. Robinson, 12 June 2003 (UF 306532, USDA 10413).

Remarks

A widespread and often abundant synanthropic species from Central America, *Beckianum beckianum* was first observed in Broward County, Florida, in 1999. Surveys in South Florida during 2002 and 2003 located additional populations and the species appears well established there. Most populations occur in leaf litter under shrubs in ditches, swamps, and other moist areas. Several of these populations are dense, with up to 50 live individuals per liter of leaf litter. Surveys conducted in the 1970s and 1980s by Florida State, Division of Plant Industries and University of Florida staff did not report this species. It is likely to have arrived in Florida within the past 20 years, probably with horticultural material from Central America or the Caribbean. This species has also been reported from Hawai'i (Cowie 2000). *B. beckianum* is not expected to be a threat from an agricultural perspective as it is a detritivore, unlike some other subulinid species, although its interaction with native species occupying similar niches remains unknown and the arrival of any alien species can lead to the introduction of diseases and parasites.

Paropeas achatinaceum (Pfeiffer, 1846)

Material examined

USA, Florida: Palm Beach County, Delray Beach, junction of Park Tree Place and Sabal Lakes Road, 26°28.9′N, 80°06.6′W, J. Slapcinsky, 15 June 2001 (UF 292691); Miami-Dade County, Miami, 861 NE 74th Street, 25°50.6′N, 80°10.9′W, F. J. Zimmerman, 8 September 2002 (USDA 10403); Homestead, University of Florida Tropical Research and Education Center, 25°30.4′N, 80°29.8′W, 2001, (UF 300999); Coral Gables, Pinewood (Cocoplum) Cemetery at Erwin and Sunset, 25°42.2′N, 80°16.0′W , D. G. Robinson, 9 June 2003 (USDA 10404); Broward County, Davie, 1 km W of Flamingo Gardens, 26°04.5′N, 80°19.4′W, J. Slapcinsky, 8 December 2002 (UF 301060).

Remarks

This small Southeast Asian species has spread across the Pacific Basin and had already reached the Hawaiian Islands by 1904 (Cowie 1997). *Paropeas achatinaceum* has not yet been recorded from the Neotropics, but it has been found in shipments of the herb *Eryngium foetidum* Linnaeus, 1753, that probably originated from somewhere in the Caribbean Basin. This species was first observed in Florida in 2001 on house foundations in Delray Beach, Palm Beach County. In the summer of 2002, several specimens were found in a bed with tropical plants at the North Carolina Museum of Natural Sciences in Raleigh, North Carolina. The plants had been imported from nurseries in Florida and it appeared likely that *P. achatinaceum* had established populations in the southern portion of that state. This suspicion was confirmed during surveys in 2002-2003 when well-established populations, most associated with horticultural plantings, were discovered in Miami-Dade, Broward and Palm Beach Counties. This species was not observed in surveys conducted in the 1970s and 1980s by Florida State, Division of Plant Industries and University of Florida staff and it is likely to have arrived in Florida within the past 20 years. Various closely-related subulinids are associated with feeding damage to roots of horticultural plants, so there is concern that the spread of this species could have repercussions for the tropical plant industry.

FAMILY BULIMULIDAE

Bulimulus tenuissimus puellaris (Reeve, 1849)

Material examined

USA, North Carolina, New Hanover County, Wilmington, Southeast Crescent container yard, 34°12.2′N, 77°57.3′W, S. Williams, 4 October 1995 (USDA 10405); S. Williams, 23 June 1999 (USDA 10406, UF 322720); D. G. Robinson, 8 September 1999 (USDA 10407).

Remarks

This Brazilian bulimulid was first detected in 1995 in a very restricted corner of the port area of Wilmington, North Carolina, and the population appears to be relatively well established, although in some years becoming quite numerous, in others the numbers dropping to almost negligible levels. The size of the population and the relative numbers of dead shells indicated that this species was introduced in the last ten years, although how it was introduced remains speculative. Because the species is believed to be a detritivore, no eradication program is contemplated, but the population is being carefully monitored by quarantine authorities to ensure that it does not spread into container storage areas that would ultimately lead to more rapid dispersal out of the port area.

FAMILY HELICARIONIDAE

Ovachlamys fulgens (Gude, 1900)

Material examined

USA, Florida: Broward County, Coral Springs, SW of junction of West Commercial Boulevard and NW 64th Avenue, 26°11.6′N, 80°14.4′W, J. Slapcinsky, 22 March 2001 (UF 288629); Coral Springs, NW of junction of Sample Road and Turtle Run Boulevard, 26°16.4′N, 80°12.8′W, J. Slapcinsky, 17 March 2002 (UF 299925); Davie, 1 km W of Flamingo Gardens, 26°04.5′N, 80°19.4′W, J. Slapcinsky and D. G. Robinson, 12 June 2003 (UF 319908); Miami-Dade County, Simpson Park at junction of South 15 Road and South Miami Avenue, 25°45.5 N, 80°11.7 W, D. G. Robinson and F. J. Zimmerman, 10 June 2003 (USDA 10415); Castellow Hammock Park, 25°33.5′N, 80°27.1′W, J. Slapcinsky and D. G. Robinson, 11 June 2003 (UF 318799); Camp Owaissa Bauer, 25°31.4′N, 80°28.1′W, J. Slapcinsky and D. G. Robinson, 11 June 2003 (UF 318790, USDA 10414).

Remarks

This small Japanese helicarionid snail (placed in the Euconulidae by Schileyko 2002) is spreading quickly in the horticultural trade throughout the tropics. It was reported from Costa Rica in 1996 (Barrientos 1998), where it is believed to have been introduced some 15 years previously, and currently is one of the most commonly intercepted snails from that country on a wide variety of plants imported for propagative purposes. It has also been intercepted by quarantine authorities on horticultural shipments and cut flowers from Thailand, Singapore, Colombia, and Trinidad and Tobago, even though it has not yet been reported by malacologists working in those particular countries. However, joint USDA/FAO surveys of pest molluscs in Trinidad conducted in January 2004 confirmed the presence of *Ovachlamys fulgens* throughout the island, although the population density was extremely low. The species was also reported from the Hawaiian islands of O'ahu and Hawai'i (Cowie 2000), as well as from the islands of Tutuila (Cowie 2001) and Olosega (Cowie *et al.* 2002) in American Samoa. The effects of this species on the environment are as yet undocumented. *O. fulgens* was first observed in Florida in 2001, where it was found under palmetto leaves along the edge of a cypress swamp. Surveys in 2002-2003 detected widespread populations of this species throughout Miami-Dade and Broward Counties in southern Florida. Individuals of this species were usually found aestivating under rocks, leaves, and wood, often near the edges of cypress swamps or in hardwood hammocks and other moist places. In October 2003, living snails were found in a greenhouse at the Lincoln Park Conservatory in Chicago (J. Gerber, pers. comm..), presumably from plants shipped from either Florida or Hawai'i. Surveys conducted in the 1970s and 1980s by Florida State, Division of Plant Industries and University of Florida staff did not report this species and it is likely to have arrived on plants, probably from Central America, within the past 20 years.

FAMILY HYGROMIIDAE

Monacha cartusiana (Müller, 1774)

Material examined

Delaware, Newcastle County, Wilmington, along railroad tracks adjacent to SICO, F. Mann and D. G. Robinson, 28 June 1999, 39°42.5′N, 75°32.1′W (USDA 10411); Port of Wilmington, 39°42.6′N, 75°32.1′W, J. Kroon and J. Bissett, 7 November 2003 (UF 306531). Port of Wilmington, 39°42.4′N, 75°32.0′W, J. Kroon and J. Bissett, 7 November 2003 (UF 306533); Illinois, Cook County, Chicago, Schiller Park, Canadian Pacific Railroad yard, 41°57.4′N, 87°52.0′W, B. Sullivan, 27 August 2001 (USDA 10412).

Remarks

A very sparse population of this widespread European snail was first reported in the port area of Wilmington, Delaware, in 1999; subsequently a much denser population was detected over a 2 ha area in a railroad yard in Chicago, Illinois, in 2001. Containers from Italy entering the country via Canada are suspected of introducing the species several years previously. We believe that the Delaware population was a secondary and probably recent introduction, either from Chicago or perhaps from another as yet undetected introduction, as the Delaware port does not handle containers from Europe. A 2003 survey found that the Delaware population, albeit sparse, extends approximately 100 ha. An eradication program of the species is being implemented, and costs have yet to be determined.

Monacha syriaca (Ehrenberg, 1831)

Material examined

USA, North Carolina, Brunswick County, Military Ocean Terminal Sunny Point (MOTSU), the "Marl Pit," 33°59.5′N, 77°57.8′W, D. G. Robinson and T. Stevens, 9-12 May 2000 (USDA 10410, UF 322722).

Remarks

Because substantial numbers of European and Middle Eastern snail species were regularly intercepted in late 1999 by quarantine authorities on military containers being unloaded at Sunny Point (MOTSU), a military depot just south of Wilmington, North Carolina, precautionary surveys of the base were conducted in early 2000. Approximately 60 ha were found to be heavily infested with the Middle Eastern snail *Monacha syriaca,* as well as isolated individuals of *Cernuella virgata* (Da Costa, 1778), *Trochoidea pyramidata* (Draparnaud, 1801), and juveniles of an unidentified *Xeropicta* species. Based on their rarity in the infested area, these other species were not believed to have established self-sustaining populations. Pest delimitation surveys determined that 60 ha had to be cleared of debris and treated repeatedly with molluscicides, at an estimated cost of $1.4 million; the eradication of all of these alien species is believed to have been successful, although the area continues to be monitored. Because the depot at Sunny Point distributes containers by rail throughout the country, other military bases that had received containers from this port had to be surveyed for alien snails. These species and individuals of other hygromiids including the Turkish *Monacha crenophila* (Pfeiffer, 1857) were found on containers at another military base in Arizona. The containers were fumigated, presumably halting the spread of these hygromiids in Arizona. However, the possibility remains that snails may have fallen from the containers anywhere along the rail lines connecting the bases. Because of the different probable origins of these species, it is also likely that introductions into Sunny Point had occurred over many years, and as-yet-undetected species may have been transported into the interior of the continent.

Xerolenta obvia (Menke, 1828)

Material examined

USA, Michigan, Wayne County, Detroit, 12594 Westwood Street, Canadian Pacific / CSX Railroad yard, 42°22.7′N, 83°13.9′W, B. Sullivan, 31 August 2001 (USDA 10408); 4 September 2001 (USDA 10409, UF 322721).

Remarks

A large and very dense population of *Xerolenta obvia* was first reported in a railroad yard in Detroit in August 2001. Delimitation surveys indicated that over 7 ha were heavily infested with this Alpine/southern European species and the density, distribution pattern, and the condition of dead shells of the snails suggested that the species had been introduced at least a decade prior to detection. Containers from southern Europe transported through southern Canada by rail are suspected of being the pathway into Michigan. The population is currently being eradicated at considerable cost, so far in excess of $100,000. Surveys in the northern Midwest and the Northeast have not detected any additional populations of this species.

CONCLUSIONS

The globalization of world trade has led to increased opportunities for alien species to be transported from almost anywhere in the world. The seven species reported here arrived via two principal pathways: (1) The Eurasian hygromiids appear to have arrived in and on shipping containers, especially those containing ceramic and marble tiles from southern Europe and those from United States military forces returning from abroad; (2) The other species of snails probably arrived on plant material imported from tropical regions, mainly from Central and South America. Those reported here were located during a few local surveys and it is probable that other alien species have been introduced elsewhere in the United States. There is clearly a need for species surveys focused on as-yet-undetected taxa, as well as for determining the ranges of previously reported species. It is likely that alien taxa remain undetected, some of which could pose serious economic and environmental costs. Workers in American malacology are encouraged to participate in molluscan surveys in their geographic area, particularly in cooperation with the USDA-sponsored CAPS program currently operating in most states nationwide. Timely surveys will allow earlier mitigation, improving the likelihood of successful eradication or control and decreasing the cost. Limiting the number of alien snail species that become established and eradicating them wherever possible may be costly, but further spread of some of these species could have serious repercussions for American agriculture and the U.S. economy as a whole. Some species, such as *Ovachlamys fulgens* and *Paropeas achatinaceum,* may have already spread to such a degree that eradication may be unfeasible. Species of Hygromiidae, such as *Monacha cartusiana, Monacha syriaca,* and *Xerolenta obvia* are generally considered to be of major quarantine significance, as they show strong invasive behavior elsewhere in the world, and members of the family are frequently serious agricultural pests. Their current distributions appear relatively restricted and eradication of these snails is therefore possible. Other taxa such as *Bulimulus tenuissimus puellaris* and *Beckianum beckianum* may be considered relatively innocuous, but nevertheless should be carefully monitored.

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Alien non-marine molluscs in the islands of the tropical and subtropical Pacific: A review*

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Abstract: This paper reviews work on aspects of the biology and management of alien non-marine molluscs in the islands of the tropical and subtropical Pacific. Excluding New Zealand, 82 land snail species (including 14 considered cryptogenic-of unknown origin) and 59 alien freshwater species (including 38 cryptogenic species) have been recorded; an additional 17 have been reported from New Zealand. Three alien freshwater bivalves have also been recorded. Larger and better-surveyed islands have more alien species recorded, with Hawaii standing apart as the most heavily invaded, having over 40 established alien snail species. A few species were introduced by Pacific islanders prior to colonization of the islands by Europeans, but the majority were introduced following the arrival of Europeans, and especially during the latter half of the twentieth century. Alien molluscs in the Pacific originate from all over the world, but, at least in Hawaii, the majority of recent introductions are of New World origin. Some were introduced deliberately, primarily as human food resources and as biological control agents. Others were introduced accidentally in association with commerce, most notably the horticultural and agricultural trades. Some species have become agricultural, horticultural, and garden pests; others prey on native snail species and have caused widespread extinctions; and others may be outcompeting native snail species. Many are able to act as vectors of parasites of humans and livestock, and some have become general nuisances. Commercial interests are affected when export shipments (e.g., of horticultural products) are rejected because of contamination by alien snails. Eradication of alien molluscs is rarely possible. Curtailing the further spread and introduction of alien snails in the Pacific depends primarily on adequate quarantine efforts, which must be implemented at three stages: pre port of entry, at port of entry, and post port of entry. At present, such efforts are inadequate or lacking. Educating the public, politicians, and business people is crucial.

Key words: Pacific, snails, alien species, biodiversity, extinction

Until recently, the non-indigenous non-marine molluscs of the Pacific had received little attention, with the notable exception of the case of the introduction of the giant African snail *Achatina fulica* Bowdich, 1822, followed by the introduction of a number of predatory species that were intended to control it (Mead 1961, 1979, Civeyrel and Simberloff 1996, Cowie 2001a). Reasons for this included the fact that over the last century or so there have been only a small number of malacologists actively working in and based full-time in the Pacific Islands. These Pacific malacologists have, quite naturally, focused their attentions primarily on the diverse native land snail fauna. Hawaii, for instance, had over 750 native land snail species, of which all but 2 to 4 were endemic (Cowie 1996b). Total native land snail diversity in the tropical and subtropical islands of the Pacific numbers probably around 4,000 species (Cowie 2004), with an additional 1,000 or more in each of New Zealand (Barker 1999) and New Guinea (Cowie 2004). Although the freshwater snail fauna of the Pacific islands is much less diverse (about 130 species, including non-native species; Haynes 2001a), it has attracted substantial scientific attention (e.g., Starmühlner 1976, Haynes 1990, 2001b and references therein). There are only 30 or so possibly native bivalve species, confined to Melanesia (McMichael 1956, Franc 1957, van Benthem Jutting 1963).

In contrast, Pacific malacologists have tended to consider the non-native species uninteresting and therefore did not collect them, record them, or study them to any great extent. This is beginning to change as the native species disappear and concern is increasing with regard to the actual and potential impacts of alien species. This review summarizes the current state of knowledge regarding these alien non-marine molluscs in the Pacific.

DISTRIBUTIONS

With the exception of a few better known islands or archipelagos, knowledge of alien non-marine molluscs in the islands of the Pacific is based on casual and incidental records rather than comprehensive and systematic survey work. Cowie (2000a, 2002a) compiled all available records of gastropods for 26 islands or archipelagos, primarily the islands of the tropical and subtropical Pacific and essentially the area covered by Motteler (1986). New Guinea was included but New Zealand and islands immediately off the coast of the Americas and Asia were not. This compilation reported 82 introduced land snail and slug species (including 14 considered "cryptogenic"—of unknown origin; Carlton 1996) and 59 alien freshwater species (including 38 "cryptogenic" species). It did not treat freshwater bivalves, three alien species of which have been recorded in the islands of the Pacific (Burch 1995, Burky *et al.* 2000), although this figure is not based on a comprehensive literature survey. Barker (1999) listed 29 alien land snail and slug species in New Zealand, of which 12 were also listed by Cowie (2000a, 2002a), giving an overall total (including those species in New Zealand) of 99 alien land snail and slug species.

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Many Pacific islands have not been surveyed adequately for native species of molluscs, and less so for aliens. New reports of alien species on particular islands, as well as new reports for the entire Pacific, continue to accumulate as further survey work is undertaken and as new aliens are introduced; usually it takes some time before these new introductions are discovered and formally recorded. Thus, the total number of alien land and freshwater molluscs in the islands of the Pacific is probably greater than has yet been recorded (see above), perhaps currently around 150 species.

Island by island distributions of these alien species, listed, as far as had then been reported, by Cowie (2002a), no doubt remain very poorly known. Major faunal surveys during the twentieth century have been few and for the most part focused on native species, for instance in Vanuatu (Solem 1959) and New Caledonia (Franc 1957, Solem 1961), although in New Caledonia Solem (1964) and Gargominy *et al.* (1996) have explicitly discussed the alien fauna. Recent field surveys and comprehensive literature compilations that have specifically included a focus on alien species have been undertaken on only a few island groups. Thus, the alien snail fauna of the Samoan islands has been well documented, based on detailed compilation of the literature (Cowie 1998a) and extensive field survey work (Cowie 2001b, Cowie and Rundell 2002, Cowie *et al.* 2002, Cowie and A. C. Robinson 2003). Specifically, this recent work in the Samoan archipelago increased the reported land snail fauna of the island of Olosega from 6 to 30 species, with the alien (including cryptogenic) species increasing from 2 to 18 species (Cowie and Rundell 2002), and that of the island of Aunu'u from 2 to 22 species, including 10 alien and 1 cryptogenic species (Cowie *et al.* 2002). Similarly, in the Hawaiian islands, a comprehensive literature survey (Cowie 1997) combined with field work and study of museum collections (Cowie 1995, 1996a, 1998b, 1998c, 1999, 2000b, Lach and Cowie 1999) has documented the alien non-marine snail fauna relatively well, with previously undocumented species being discovered at a rate of around one every year (Cowie 1998b, Cowie and D. G. Robinson 2003).

Although there may be only about 150 species of alien non-marine molluscs in the Pacific islands, in contrast to probably about 5,000 native species (perhaps 6,000 if New Zealand is included), some of these alien species are extremely widespread, whereas the vast majority of the native species are single island or single archipelago endemics (e.g., Cowie 1996b). Nevertheless, most of the alien species occur on just a few islands, and some islands appear to have none, probably because they have not been adequately surveyed recently. The larger and/or better studied islands seem to have the largest numbers. For instance, 49 of the 82 alien land snail/slug species so far recorded occur on only one to three islands or island groups (excluding those found only in New Zealand), with a large number (19) of these only in Hawaii. These patterns have been discussed more fully by Cowie (2002a).

A few of these land snail species, however, are widely distributed. These include a mixture of cryptogenic species that may have been transported widely by Pacific island people in prehistoric times (e.g. *Lamellidea oblonga* [Pease, 1865], *Lamellidea pusilla* [Gould, 1847], and *Liardetia samoensis* [Mousson, 1865]), species considered definitively to have been introduced by Pacific islanders prior to Western exploration of the Pacific (*Allopeas gracile* [Hutton, 1834]), and more recent introductions (*Achatina fulica, Euglandina rosea* [Férussac, 1821], *Subulina octona* [Bruguière, 1789], and *Bradybaena similaris* [Rang, 1831]).

GEOGRAPHICAL AND TEMPORAL ORIGINS

The majority of the 68 non-cryptogenic species of land snails reported by Cowie (2000a, 2002a) are modern introductions, that is, since Western discovery and colonization of the islands of the Pacific, as are all the species reported from New Zealand by Barker (1999). Among the freshwater snail species, all those that are definitively considered alien in the Pacific are also modern introductions. Among the alien bivalves, *Corbicula fluminea* (Müller, 1774) is of recent Asian origin (Burch 1995), and one of the two sphaeriids, *Musculium partumeium* (Say, 1822), is a North American species (Lee and Ó Foighil 2003) and probably a recent introduction, although Burky *et al.* (2000) suggested that sphaeriids might have been continuously introduced throughout the early history of human colonization of the Pacific.

By definition, however, the origins of the cryptogenic species are unknown. Most of the cryptogenic land snails appear to be Pacific island endemics that are widespread throughout the region, and it is their origins on particular islands that are obscure. These include five achatinellid species, three helicarionids, five pupillids, and one zonitid (Cowie 2002a). At least some of these may have been transported inadvertently around the region by Pacific islanders prior to Western colonization. The cryptogenic freshwater species, however, include, notably, 31 thiarids. Many of these have been considered native to the Pacific (Haynes 1990). The taxonomy of these thiarids has been little studied (references in Cowie 1998a); many nominal species may be junior synonyms described on the basis of minor variations in shell characters that can easily arise in colonizing populations of these apparently primarily parthenogenetic snails (Haynes 1988). The cryptogenic freshwater snails also include four planorbids, a hydrobiid, and two ancylids (Cowie 2002a). The true identities of the ancylids are questionable (Hubendick 1967), as are those of the planorbids. The origin of the hydrobiid, which bears a striking resemblance to North American species of *Tryonia* but has been found in archaeological deposits that pre-date Western colonization, is enigmatic (Cowie 1998b). The sphaeriid bivalve *Pisidium casertanum* (Poli, 1791) is cosmopolitan and may include a number of cryptic species (Lee and Ó Foighil 2003). Its geographic origins in the Pacific are unknown; it may be a modern introduction or may have been transported around the Pacific by early Pacific islanders (Burky *et al.* 2000), or it may even be indigenous in the Pacific given that sphaeriids are readily transported by birds (Mackie 1979).

The geographic origins of the snail and slug species introduced to Hawaii have been discussed in detail by Cowie (1998b), with numbers updated by Cowie and D. G. Robinson (2003). In total, 85 species are known to have been introduced up to 1999, although not all of these became established. Briefly, three species were introduced by Pacific islanders prior to Western colonization of the islands. From 1778, when the first Westerner (Captain Cook) arrived in Hawaii, to 1909, 15 of the 20 introduced species for which a geographic origin was known came from Australasia or the Americas, that is, the Pacific Rim. During the period 1910-1999, with the advent of faster shipping and subsequently air travel, and with the annexation of Hawaii by the U.S. and dramatically increased commerce with the U.S., New World species began to dominate the introductions (23 of the 48 species of known origin), superimposed on an overall increase in total number of species introduced from all over the world. In the 1950s and 1960s a large number of African predatory species were introduced in attempts to control the previously introduced giant African snail, *Achatina fulica*. Following a more focused effort during the 1990s to document new introductions, and with increased trade between Hawaii and Asia, especially horticultural trade, the number of recorded introductions jumped; notably a number of Asian species were introduced, including *Parmarion martensi* Simroth, 1893, *Ovachlamys fulgens* (Gude, 1900), and *Liardetia doliolum* (Pfeiffer, 1846) (Cowie 1997, 1999, 2000b).

The introduced land snails and slugs of New Zealand have been treated in great detail by Barker (1999). Of the 29 introduced species (Table 1), most are of western Palearctic origin (especially if the two Holarctic species are included in this category), clearly reflecting New Zealand's strong ties to Europe, especially during its early settlement by Westerners, and its largely temperate climate. Only three species are Nearctic in origin; two are Holarctic; one, *Deroceras laeve* (Müller, 1774), is a cosmopolitan species considered by Barker (1999) as of unknown geographic origin, although most likely Palearctic or perhaps Holarctic; and one, *Coneuplecta calculosa* (Gould, 1852), is a widespread Pacific island species. Notably, none is Asian. Just over half of these species were first recorded in New Zealand during the nineteenth century (Table 1).

The introduced land snails and slugs of New Caledonia (Gargominy *et al.* 1996) have more diverse origins (Table 2), although in this case dominated by New World species and with a greater complement of Pacific species, perhaps reflecting the tropical location of New Caledonia as opposed to the subtropical/temperate location of New Zealand. But despite the strong cultural, economic, and political connections to Europe, only a single European species (*Helix aspersa* Müler, 1774) has been recorded. Again, just over half the species were first recorded during the nineteenth century.

Overall, the introduced snails in the Pacific (excluding New Zealand) come from all over the world. They are dominated, as would be expected, by species from the New World and Asia/Australasia, but there are significant numbers also from Europe and Africa (Cowie 2002a). New Zealand is dominated by European species. Particular islands or archi-

Table 1. The origins of the introduced land snails and slugs of New Zealand (data compiled from Barker 1999).

Region of origin	19th century	20th century	Total
W. Palearctic	14	8	22
Nearctic	—	3	3
Holarctic	2	—	2
Pacific	—	1	1
Cosmopolitan	—	1	1
Total	16	13	29

Table 2. The origins of the introduced land snails and slugs of New Caledonia (data compiled from Gargominy *et al.* 1996).

Region of origin	19th century	20th century	Total
Europe	1	—	1
New World	6	6	12
Holarctic	1	—	1
Pacific	4	1	5
Africa	1	1	2
Asia/Australasia	—	3	3
Total	13	11	24

pelagos have a subset of the total alien fauna, often reflecting their location, climate, and history of human colonization, as has been shown above for Hawaii, New Zealand, and New Caledonia. In contrast to the temporal trends in the geographic origins of the introductions in Hawaii, no clear trends appear when the Pacific is analyzed as a whole (Cowie 2002a), perhaps because the numbers are small, but perhaps also because of the great complexity of international relationships that connect the Pacific (e.g., Carlton 1987). However, the rate of introductions has more than tripled in the twentieth century: excluding New Zealand, 65 non-cryptogenic species were introduced to the islands of the Pacific compared to 20 species prior to 1900 (Cowie 2002a).

PATHWAYS OF INTRODUCTION

The worldwide pathways by which alien non-marine molluscs are transported have been discussed in detail by Cowie and D. G. Robinson (2003). Probably molluscs have been and continue to be moved around the Pacific via most of these pathways. However, few data exist documenting this. Of the 235 records of 68 alien species established on 26 islands or island groups across the Pacific (not including New Zealand), only 42 records can be more or less definitively associated with a particular single pathway; a few are known to have been introduced via more than one pathway or for more than one reason; but the pathways of introduction of the great majority are unknown (Cowie 2002a, Cowie and D. G. Robinson 2003). The following summarizes the known and likely pathways of introduction in the Pacific; other pathways have been documented elsewhere in the world, or can be readily hypothesized (Cowie and D. G. Robinson 2003); some may only be important for extending the distribution within an island of species already introduced to the island. Introductions are either deliberate or inadvertent.

Deliberate introductions

Aquarium industry: Freshwater snails are imported deliberately for sale in pet stores for use in domestic aquariums and may escape or be released.

Food (includes aquaculture): Various species of terrestrial and freshwater snails introduced as "escargot" or for local ethnic consumption, including *Helix aspersa, Achatina fulica, Pomacea canaliculata* (Lamarck, 1822), and *Cipangopaludina chinensis* (Griffith and Pidgeon, 1834), have either been deliberately introduced to the wild or have escaped (van der Schalie 1969, 1970, Gargominy *et al.* 1996, Cowie 1997, 1998b).

Medicinal purposes: The giant African snail, *Achatina fulica,* was introduced to Hawaii for unspecified medicinal purposes, as well as for other reasons (van der Schalie 1969, 1970).

Biological control: Predatory snails, notably *Euglandina rosea* and *Gonaxis* spp., but also a large number of other species, have been introduced widely in the Pacific in ill-conceived attempts to control *Achatina fulica.* There is no good evidence that the predatory snails reduce populations of *A. fulica* but there is ample evidence of their devastating effects on native snail faunas (reviewed by Civeyrel and Simberloff 1996, Cowie 2001a).

Aesthetics: One of the original introductions of *Achatina fulica* into Hawaii was for ornamental purposes (van der Schalie 1969, 1970).

Biological research: Three species of *Cerion* were released in Hawaii in the 1920s to investigate the relative importance of genetic and environmental control of shell morphology but none became established (Cowie 1996a).

Inadvertent introductions

Snails and slugs are transported in or on a huge range of products, conveyances, containers, etc. Some species probably travel most readily as eggs or small juveniles. The following list is probably not comprehensive but highlights the most likely major pathways of inadvertent introduction in the Pacific.

Agricultural products (excluding horticulture): Of 237 records of snails and slugs intercepted by the State of Hawaii Plant Quarantine Branch (14 December 1994 to 6 August 1999), 164 (69%) were associated with agricultural products (N. Reimer, pers. comm.; Cowie and D. G. Robinson 2003). Of 22 records of snails and slugs intercepted entering New Zealand from 1955 to 1978, ten (45%) were associated with agricultural products (Barker 1979, Cowie and D. G. Robinson 2003).

Horticultural products: Cut flowers, live plants, seeds, turf, leaves used for mulch, potting soil and other media (see also below), etc. provide a ready pathway for introduction. Many nurseries are infested with snails and slugs, especially small species (e.g., in Hawaii; Hara and Hata 1999). Some species have been imported to nurseries from elsewhere and then re-exported to a further region (e.g., *Liardetia doliolum,* alien in Hawaii but exported to the U.S. mainland from a nursery in Hawaii; Cowie 1999). Often, new records of alien species in an area are associated with nurseries, garden stores, botanical gardens, or recent landscaping activities (e.g., *Parmarion martensi* and *Polygyra cereolus* (Mühlfeld, 1818) in Hawaii; Cowie 1998c).

Commercial and domestic shipments: In general, any number of commodities or household goods, usually but not always shipped in containers, may have snails and slugs associated with them (Robinson 1999). These may be inadvertently transported from the source with the goods themselves, or may attach themselves to the goods, their packaging, or the shipping container at any point en route. Data from New Zealand (Barker 1979) and Hawaii (N. Reimer, personal communication) suggest that commercial and domestic shipments are a less important pathway than agricultural and horticultural trade, but this may reflect the relative levels of inspection of different categories of goods by quarantine officials (Cowie and D. G. Robinson 2003).

Military shipments: Snails and slugs may also be transported with military supplies and other goods associated with military campaigns, as well as during routine peacetime transportation of military equipment and supplies. Hawaii and Guam, for instance, are involved in considerable U.S. military activity.

Soil and other growth media: Snails and slugs, and especially their eggs, are readily transported in soil and other growth media (e.g., moss). Soil can be deliberately or accidentally transported with agricultural or horticultural products (e.g., potted plants), or on vehicles, shoes, etc. This may be a minor pathway in the Pacific; it is prohibited to import shipments with soil or growth media into Hawaii, but recent efforts have been made to relax this regulation to allow moss to be imported with orchids.

Aquarium industry: Small freshwater snails, including thiarids, physids, lymnaeids, and planorbids, are easily transported inadvertently attached to aquarium plants and in association with aquarium and other alien fish. They may be released when the aquarium or its contents is discarded.

Aquaculture: Aquatic snails may be introduced accidentally (or even intentionally) along with the species (not necessarily snails) specifically introduced for aquaculture (both for food and for the aquarium industry).

Ships/boats: If a ship takes on ballast water in a freshwater harbor and then discharges it in another freshwater harbor, freshwater snails (or their larvae) could be introduced. However, there are no freshwater harbors in the Pacific islands, so this pathway is probably only important in the Pacific for marine and brackish-water molluscs. Dry ballast is no longer used and thus no longer constitutes a pathway for introduction of terrestrial species. However, ships and small private craft (e.g., inter-island yachts) may also be the inadvertent vehicles transporting terrestrial snails that are accidentally brought onboard.

Airplanes: Snails may attach themselves to airplanes but their likelihood of survival on long-distance, high altitude flights is low. Nevertheless, airplanes may be involved in relatively short-distance dispersal, including dispersal between nearby islands. Seaplanes may be similarly involved in dispersal between lakes.

IMPACTS

The impacts of alien molluscs in the islands of the Pacific, as elsewhere, can be categorized as ecological (including native biodiversity), agricultural/horticultural, relating to human and livestock health and well-being, and commercial (Staples and Cowie 2001). These categories are not mutually exclusive and may overlap in some cases, and some alien species may have multiple impacts.

Environmental impacts

Predation: Perhaps the most well-known and related stories of introduced non-marine snails in the islands of the Pacific are those of *Achatina fulica* and *Euglandina rosea.* In 1936, the giant African snail, *A. fulica,* was introduced to Hawaii from Japan (Cowie 1997). By the 1950s it had become so abundant that efforts were initiated to try to control it by introducing predatory snails as biological control agents. In short, 15 predatory snail species were introduced into the wild in Hawaii; a small number of them became established, the most notable of which is *Euglandina rosea* (Cowie 1998b). There is no good scientific evidence that *E. rosea* (or any of the other predatory snails) has controlled populations of *A. fulica* in Hawaii, but *E. rosea* has been heavily implicated in the decline and possible extinction of Hawaiian tree snails (Achatinellinae) (Hadfield 1986, Hadfield *et al.* 1993) and possibly of other endemic Hawaiian snails. *Euglandina rosea* (and some of the other predatory species) has been taken to other Pacific islands, notably the Society Islands of French Polynesia (Tahiti, Moorea, etc.), where it has caused similar decline or extinction, especially of the Partulidae (Murray *et al.* 1989, Coote and Loève 2003) but probably also of other endemic species. This sequence of events and its consequences have been reviewed in greater detail by Civeyrel and Simberloff (1996) and Cowie (2001a).

Competition: There are no clear cases in the Pacific islands in which competition between introduced and native species of molluscs has been demonstrated. However, Cowie (2001b) has speculated that the decline of certain litter-dwelling snail species in the Samoan islands (e.g., *Pleuro-*

poma fulgora [Gould, 1847]), and perhaps elsewhere, has been caused by competition between these species and introduced members of the Subulinidae, notably *Allopeas gracile, Subulina octona,* and *Paropeas achatinaceum* (Pfeiffer, 1846).

Habitat modification: Again, there is no documented evidence for this, but anecdotal speculation (e.g., Civeyrel and Simberloff 1996) suggests that introduced species such as *Achatina fulica* and veronicellid slugs (e.g., *Laevicaulis alte* [Férussac, 1822], *Veronicella cubensis* [Pfeiffer, 1840], and *Vaginulus plebeius* [Fischer, 1868]) may be preventing rainforest regeneration by destroying newly germinated seedlings of native forest plants.

Agricultural and horticultural impacts

Introduced freshwater apple snails (Ampullariidae), especially the species currently identified as *Pomacea canaliculata,* were brought to Hawaii as potential human food resources. They were released into taro fields (similar to rice paddies) and are now the most serious pest of taro (Cowie 2002b). This species has also been reported in Guam (Smith 1992) and New Guinea (Eldredge 1994). The North American land snail *Zonitoides arboreus* (Say, 1819) is a pest in orchid nurseries in Hawaii (Hara and Hata 1999). Other species have been considered agricultural and garden pests in the Pacific, especially *Achatina fulica,* although Civeyrel and Simberloff (1996) suggested that its agricultural impacts had been over-emphasized. Various other snails (e.g., *Bradybaena similaris*) and veronicellid slugs (personal observations) have also been considered pests.

Human and livestock health and well-being

Many snails and slugs act as vectors of human and animal parasites (Alicata 1969). Most notable among these parasites are the rat lungworm, *Angiostrongylus cantonensis* (Chen, 1935), which causes eosinophilic meningoencephalitis in humans, and the cattle liver fluke *Fasciola hepatica* (Linnaeus, 1758). Many snails that have been tested appear able to act as vectors of *A. cantonensis,* although the level of infection varies widely among species, with larger and terrestrial species being more susceptible (Wallace and Rosen 1969a, b). However, the human health threat posed at least by *Achatina fulica* acting as a vector of *A. cantonensis* may have been exaggerated (Civeyrel and Simberloff 1996). The major hosts of cattle liver flukes in Hawaii are the lymnaeids *Fossaria viridis* (Quoy and Gaimard, 1832) and *Pseudosuccinea columella* (Say, 1817) (numerous references listed by Cowie 1997). Presumably, native snail species would also be potential vectors of these parasites, but because many of these native species are extinct, confined to high elevation refugia, or are highly localized on many Pacific islands, it is the alien species that are now the major vectors.

Some species have simply become a general nuisance. *Achatina fulica* became so abundant in the 1950s in Hawaii that it was almost impossible to avoid them when walking on the sidewalks. They would crawl up the sides of houses in great numbers, and there were even stories of cars skidding on them (Mead 1961).

Commerce

Probably the most important commercial impact of introduced snails and slugs, apart from their direct impacts on commercial agricultural and horticultural production and the consequent economic costs of implementing control measures, is that they may often be contaminants of shipments of these products. This can lead to rejection of a shipment exported to another jurisdiction. For instance, the first knowledge that *Liardetia doliolum* was present in Hawaii was its interception by quarantine authorities in California (and shortly thereafter in Arizona) on shipments of horticultural products exported from Hawaii (Cowie 1999).

MANAGEMENT

A more comprehensive account of the needs for management of alien non-marine molluscs in the Pacific, which has wider applicability than simply to molluscs, has been presented by Cowie and D. G. Robinson (2003), summarized by Cowie (2004), and briefly touched on by Cowie (2002a) and Lydeard *et al.* (2004). The essential points are as follows.

Eradication and control

Eradication of alien species may sometimes be possible (Myers *et al.* 2000). In the Pacific, new infestations of *Achatina fulica* have been eradicated in Tuvalu and possibly Kosrae (Anonymous 1996, 1998), and eradication of a new infestation of the freshwater snail *Pila conica* (Wood, 1828) (and perhaps of *Pomacea* sp.) was accomplished in Palau (Eldredge 1994, Cowie 2002b).

Eradication of well-established aliens is much more difficult. Nevertheless, efforts have been made to eradicate and then exclude well-established alien predatory snails (and rats) from small (approximately 500 m^2) snail reserves in Hawaii (Olival and Hadfield 2000) and on Moorea in French Polynesia, although with limited success largely because of the difficulty of maintaining the exclosures (Murray 1996, Clarke and Pearce-Kelly 1997).

Control or management of alien snails, as opposed to complete eradication, may be possible, but again perhaps only on a small scale. As yet, no successful programs have been developed and widely implemented in the Pacific. Major efforts have been made in attempts to control *Achatina* *fulica*, especially focusing on biological control with predatory snails. But these efforts have been disastrous, as noted above. Nor have other methods of managing *A. fulica* been generally successful on a large scale or over the long term (e.g., use of molluscicides, hand collecting). In Hawaii a bait has been developed against the predatory snail *Euglandina rosea* (Hadfield 1998), but has been used only experimentally because of permitting issues (M.G. Hadfield, pers. comm.).

Prevention of further introduction and spread

Preventing entry of a harmful species is always preferable to attempting to eradicate or control it after it has been introduced. Prevention fundamentally requires aggressive quarantine regulations and actions at three basic stages, as follows.

Pre-entry regulations and screening: Because there will always be a demand for certain species, it is not possible simply to prohibit the importation of all species. Therefore, lists are developed either of species that are not permitted to be imported because they are known or thought likely to become invasive and cause problems ("blacklists") or of species that are permitted to be imported because they are considered unlikely to cause problems ("whitelists"). The latter are preferable as they are more restrictive. Screening protocols, such as those developed for plants by a number of countries, would allow authorities to evaluate the likely invasiveness of an alien species of mollusc and whether it should be placed on one of these lists. The advantages, pitfalls, and likelihood of implementation of these possible approaches have been discussed in more detail by Cowie and D. G. Robinson (2003).

Port-of-entry quarantine (large and small scale): This can be at the level of a country, a subdivision of a country (state, county), a group of islands, a single island, or even smaller areas such as a reserve. It requires far more personnel, who must be adequately trained, than are currently employed even in wealthy countries.

Post-entry rapid response (eradicate new populations): The need to quickly eradicate new populations that result from alien species slipping through the first two stages of the quarantine net has been discussed above.

No Pacific island nation (or other political jurisdiction) has yet implemented such a rigorous and adequate tripartite quarantine protocol.

Finally, and although it is becoming something of a refrain, educating and influencing not only the general public but also politicians, managers, and business people about the impacts, realized and potential, of alien species is crucial. While it is important that malacologists, as well as other biologists, communicate with their colleagues by publishing in their disciplinary, peer-reviewed journals, it is in the end more important to air their concerns much more widely (Ponder 2004).

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A South American bioinvasion case history: *Limnoperna fortunei* (Dunker, 1857), the golden mussel*

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Abstract: Two factors combine in this age of globalization to favor the establishment of alien species in natural environments: human activities and global climate change. This paper reviews the recent invasion of the golden mussel, *Limnoperna fortunei,* in South America, including its impacts in natural and human environments. This case study allows the identification of the likely impacts that morphologically and functionally similar invasive species will have in similar environments, such as in North America, which is considered to be at high risk of invasion by *L. fortunei.*

Key words: Mytilidae, biological invasion, South America, macrofouling

Two factors combine in this age of globalization to favor the establishment of alien species in natural environments. First, through intentional or non-intentional activities of people, non-native species are introduced into and distributed within areas beyond their natural historical range (Johnson and Carlton 1996, Ricciardi and MacIsaac 2000, Ruiz *et al.* 2000). Second, global climate change may result in new regions becoming potentially available for colonization, especially for those species able to adapt to the newly accessible environment (Leach 2000).

Many human activities, including agriculture, aquaculture, recreation, transportation, the aquarium trade, and construction of canals and other aquatic diversions, promote the spread of species beyond their natural dispersal barriers (Ruiz *et al.* 1997, Benson 2000). This entails alterations in the composition and functioning of ecosystems (Grosholz 2002). Although there are many mechanisms for non-intentional introduction of species, for aquatic species, discharge of ballast water in foreign waters is recognized as the most common cause of such introductions (National Research Council 1996, Mackie 2000, Fofonoff *et al.* 2003 [and references therein]). Carlton and Geller (1993) described the increasing introduction of aquatic species via the ballast water of cargo ships as "ecological roulette."

Human activities during the last decades of the twentieth century resulted in the emission of substantial amounts of anthropogenic greenhouse gases. However, these may not be solely responsible for the steep rise in the earth's temperature and may only be accelerating a process of natural change (Broecker 2001). Two global events strongly influenced biotas during the past millenium: the Medieval Thermal Maximum (800-1200 A.D.) and the Small Ice Age (1450-1850 A.D.) (Jones *et al.* 2001, Deschamps *et al.* 2003). Dispersal of a species into a region beyond its existing range, as well as its establishment and adaptation within the new range, are due not only to chance but also depend on the presence of appropriate environments within the new region. Climate change may enhance the invasibility of such regions. Global climate change affects the distribution of species as well as resource dynamics in both terrestrial and aquatic ecosystems, thereby modulating biological invasions. Stachowicz *et al.* (2002) relate the establishment of introduced ascidean species in New England to the increase in winter water temperatures from the 1970s to the 1990s. Dukes and Mooney (1999) mentioned that increased CO_2 levels might slow the process of succession in grasslands, which would increase the dominance of non-native species in many ecosystems. They also suggested that the temperature increase, resulting from climate change, might benefit the Argentine ant (*Linepithema humile* [Mayr, 1868]) in its invaded area to the detriment of native ant species. Likewise, both the increased global transport of species and increased coastal ocean temperatures in the past few decades may provide an explanation for the increasing rate of invasion by alien species (Stachowicz *et al.* 2002).

Within these general contexts (invasion and global change), Dukes and Mooney (1999) addressed whether some ecosystems are more or less prone to invasion than others, whether certain alien species will become invasive, and whether the impacts caused by invading species will be severe or mild. Integration of theoretical principles with the scarce studies on how biological invaders respond to global change led to the conclusion that the most important elements of global change seem to increase the prevalence of biological invasions.

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From an agricultural point of view, the Argentine pampas is one of the most productive areas in South America. One of its main waterways, the Río de la Plata, was the entrance point – during the past three decades – of at least three alien molluscan species, i.e., *Corbicula fluminea* (Müller, 1774), *Limnoperna fortunei* (Dunker, 1857) (Darrigran 2002), and *Rapana venosa* (Valenciennes, 1846) (Pastorino *et al.* 2000); the first two have already been acknowledged as invasive in the region. This may be an example of an invasion involving not only human maritime activity (through the port of Buenos Aires) but also, although highly speculatively, a global climate change that took place during the past few decades. This climate change included a 100% increase in rainfall in the region (Fig. 1) (Deschamps *et al.* 2003), an alteration that carried manifold consequences for freshwater ecosystems. Enhanced commercial links during recent decades, together with rapid climatic change, could have facilitated the arrival and establishment of the three alien species.

Dukes and Mooney (1999) also commented on the feedback interaction connecting the impacts of new invading species on the environment, global change, and human action through commerce on a global scale (Fig. 2). All this points to a future in which "foreign ecosystems" will be the rule. Single elements of global change might affect biological invasions. For instance, the rising concentration of carbon dioxide could increase the dominance of invaders in some ecosystems. Climate change might favor the many alien species that can shift range quickly and/or tolerate a wide range of environments. Land-use patterns that increase habitat fragmentation can increase the prevalence of non-native species. The interactions among the elements of global change that affect the prevalence of biological invaders remain unstudied.

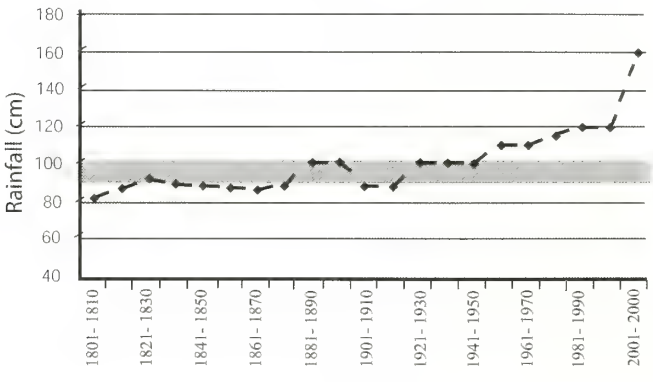

Figure 1. Rainfall in Buenos Aires from 1801 to 2002 (modified from Deschamps *et al.* 2003). In gray, range used for public works planning at the end of the 19th century and for much of the 20th century.

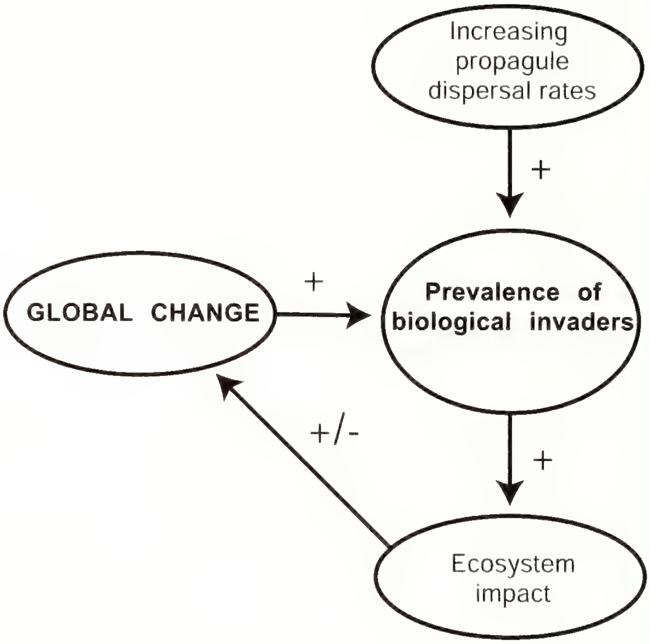
Increasing propagule dispersal rates
+
GLOBAL CHANGE
+
Prevalence of biological invaders
+/-
+
Ecosystem impact

Figure 2. Forces that may affect the prevalence of invasive species. Elements of global change and changes in global commerce increase the rate of arrival of alien species. The invader species alter ecosystem processes and properties; many of them may interact (positively or negatively) with elements of global change (modified from Dukes and Mooney 1999).

Levine and D'Antonio (2003) suggested that the ecological and economic costs associated with human-caused biological invasions may continue to rise substantially over the next decades. Numerous international meetings organized by diverse organizations have had the underlying aim of preventing the realization of such predictions. Two such meetings, focused on molluscs, were recently held: a workshop on "Freshwater invading bivalves in southern South America" during the Fifth Latin American Malacological Congress (São Paulo, Brazil, 2002) and the symposium during the 69th Annual Meeting of the American Malacological Society (Ann Arbor, USA, 2003) to which this paper is a contribution. Independently, similar discussions arose and the same conclusions were drawn at these meetings, essentially: (1) there is a lack of public awareness of the problem; (2) this leads to a lack of control over human activities that cause introduction/dispersal of foreign species; and (3) there is a general lack of interest in generating knowledge to prevent the economic/environmental problems at a regional scale.

In order to establish *a priori* criteria for the prevention or treatment of an invasion – knowing that studies on populations in their natural ranges are inadequate – patterns of impact for species with an invasion history should be identified. This could lead to the ability to predict the impacts of morphologically and functionally similar species in similar environments (Bij de Vaate *et al.* 2002, Ricciardi 2003). Comparative studies of these patterns, which serve as major sources of understanding of the processes involved in invasion capacity and the consequences of invasions, require the collection of comparable data sets (Orensanz *et al.* 2002). However, currently available quantitative data on the history of bioinvasions are inadequate for comparison of similar ecosystems (Ricciardi 2003). Although the number of scientific publications on bioinvasions has increased over the past decade (Kolar and Lodge 2001), Orensanz *et al.* (2002) concluded that the literature on estuarine or marine invasions is unevenly distributed, with a majority from the USA, Western Europe, and Australia. Much of the little that is known about the issue in South America is either unpublished or reported in relatively obscure sources not normally available to the international scientific community (Orensanz *et al.* 2002).

An alternative approach to predicting the impact of an introduced species is to infer it from the invasion history of functionally similar organisms. Therefore, knowledge of the biology of invasive bivalves such as *Dreissena polymorpha* (Pallas, 1771), *Limnoperna fortunei, Mytilopsis sallei* (Récluz, 1849), *Modiolus striatulus* (Hanley, 1853), *Perna viridis* (Linneaeus, 1758), and *Xenostrobus securis* (Lamarck, 1819) could serve as a template to set priorities in the study of other potential pest bivalves that do not yet have an invasion history (Ricciardi 2003).

This paper presents a synthesis of a bioinvasion case history: the invasion of *Limnoperna fortunei* in South America. Enhanced knowledge about this species may be of value in predicting and preventing potential impacts of invasions by bivalve species with similar morphological and functional features.

LIMNOPERNA FORTUNEI: A CASE HISTORY

Numerous alien species have arrived in the Americas during the recent past. Among them are three freshwater bivalves that became invasive: *Corbicula fluminea* (Asian clam), *Limnoperna fortunei* (golden mussel, Fig. 3), and *Dreissena polymorpha* (zebra mussel). The zebra mussel has been present in North America since the 1980s but is absent from the Neotropical Region (Ricciardi 2003).

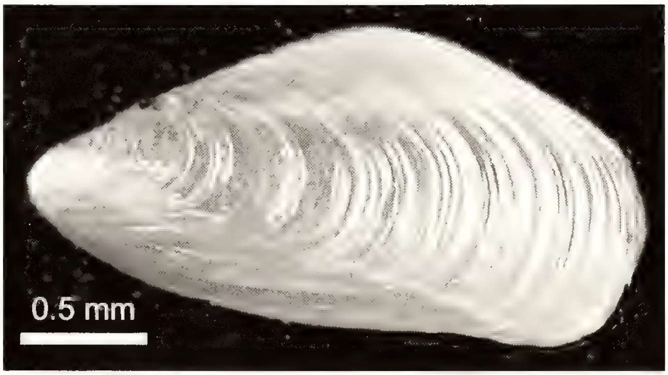

Figure 3. External view of left valve of *Limnoperna fortunei.*

The golden mussel, *Limnoperna fortunei,* is native to southeast China and Korea (Iwasaki and Uryu 1998). It was introduced to Hong Kong in 1965 (Morton 1977) and to Japan (Kimura 1994) and Taiwan (Ricciardi 1998) in the 1990s. It was found for the first time in the Americas in 1991 in the Argentine pampas at Bagliardi Beach, Río de la Plata estuary (35°55'S, 57°49′W) (Pastorino *et al.* 1993) (Fig. 4), having been introduced in the ballast water of ocean-going vessels (Darrigran and Pastorino 1995).

Several factors enabled *Limnoperna fortunei* to spread rapidly and to have a severe impact on natural and human environments in South America, including its short life span, rapid growth, early sexual maturity, high fecundity, ability to colonize a wide range of habitat types, wide range of physical tolerances, gregarious behavior, suspension feeding, and planktonic larvae (Morton 1996). In addition, the South American native freshwater bivalves, which include unionids, corbiculids, and sphaeriids, are predominantly infaunal in soft substrates and lack planktonic larvae. Thus there was an open opportunity for the epifaunal, hard substrate-dwelling alien (Darrigran 2002). Because its initial introduction was in an industrialized area with heavy river trade, it was immediately associated with human activity, settling on surfaces of commercial and/or recreational ships and boats, on artificial structures such as piers and wharves, in pipes supplying water for industry, and in water purification plants, refrigeration systems, etc. These characteristics have rendered *L. fortunei* the environmentally and economically most harmful freshwater invading bivalve in South America, similar to *Dreissena polymorpha* in North America.

Distribution and dispersal

The Río de la Plata is part of one of the major hydrographic systems in South America, the Plata basin. This system covers a drainage area of 3,000,000 km^2 (Fig. 4), with the richest freshwater molluscan diversity of the Patagonian

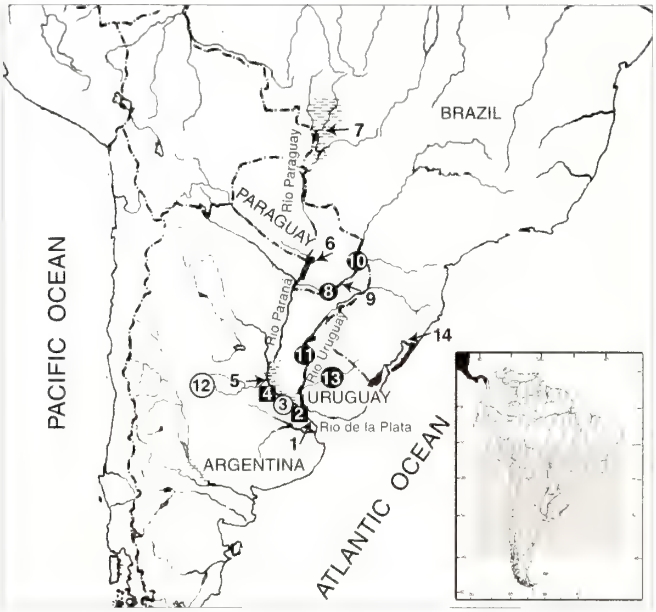

Figure 4. Distribution of *Limnoperna fortunei* in South America. 1, Bagliardi Beach (Argentina); 2, Buenos Aires Harbor (Argentina); 3, Atucha (Argentina); 4, San Nicolás (Argentina); 5, Santa Fe (Argentina); 6, Asunción Harbor (Paraguay); 7, The Pantanal (Brazil-Bolivia); 8, Yacyretá (Argentina-Paraguay); 9, Posadas Harbor (Argentina); 10, Itaipú (Brazil-Paraguay); 11, Salto Grande (Argentina-Uruguay); 12, Río Tercero (Argentina); 13, Río Negro (Uruguay); 14, Guaiba Basin (Brazil). Black circles, hydroelectric power plants; black squares: thermal power plants; white circles, nuclear power plants.

subregion of the Atlantic slope (Darrigran and Pastorino 2004) and good navigability in some of its rivers (Paraná, Río de la Plata, Paraguay), which sustain intense commercial traffic.

Since its first appearance in the Río de la Plata in 1991, the distribution of *Limnoperna fortunei* has been constantly expanding. In 1994 it was present only in the Río de la Plata, on both the Argentine and Uruguayan coasts (Scarabino and Verde 1994). Since 1995 it has been recorded in the industrialized area along the lower Paraná River, causing the earliest cases of macrofouling (Darrigran 1995). Two years later it was recorded in Asunción (Paraguay) on the Paraguay River. In 1998 it was collected in the Pantanal (Dreher Mansur *et al.* 2003), a World Ecological Sanctuary. It continued its northward dispersal along the Paraná River and in 1998 was recorded in Posadas and at the Binational Hydroelectric Power Plant at Yacyretá (Darrigran and Ezcurra de Drago 2000). By 2001 it had reached the Binational Hydroelectric Plant of Itaipú (Darrigran 2002, Zanella and Marenda 2002). It also dispersed up the Uruguay River (I. Ezcurra de Drago pers. comm.), being recorded in 2001 at the Binational Hydroelectric Power Plant of Salto Grande. Also in 2001, it was recorded in the reservoir and at the nuclear power plant in Río Tercero, Córdoba, Argentina (unpublished data). This large region encompasses widely varying environmental conditions and temperate to subtropical climates.

Limnoperna fortunei was also detected in Río Grande do Sul (Brazil) in November 1999 (Dreher Mansur *et al.* 1999). This is considered to be the outcome of a separate invasion event, probably resulting from commerce between Argentina and Brazil (M. C. Dreher Mansur pers. comm.). In Brazil it has invaded the states of Río Grande do Sul, Paraná, Mato Grosso, and Mato Grosso do Sul (R. J. Calixto pers. comm.). Several of these areas sustain important industries and their water cooling systems have been invaded by *L. fortunei* or are under imminent threat of invasion.

Planktonic larvae provide the natural dispersal mechanism of *Limnoperna fortunei*. However, in contrast to the spread of *Dreissena polymorpha* in North America (Mackie and Schloesser 1996), in the Plata basin *L. fortunei* dispersed mainly in a countercurrent direction, at an average rate of 240 km/yr (Darrigran 2002). This rapid countercurrent dispersal is probably related to human activities including commerce, fishing, and recreation, which are enhanced by the navigability of the rivers.

In South America, *Limnoperna fortunei* first occupied environments with water temperatures of 14-24°C. At a later stage it invaded areas with shorter winters and temperatures of 15-33°C. It can also inhabit brackish waters (salinity <3 ppt), and tolerates pH values of 6.2-7.4 and Ca^{++} concentrations of 3.96 mg/l (Darrigran 2002).

Density

When first detected in the Río de la Plata, the density of *Limnoperna fortunei* was 4-5 individuals per m^2 (Darrigran and Pastorino 1995, Darrigran 2000). Density peaked at the same locality in 1995 with ~150,000 per m^2, subsequently stabilizing at ~40,000 per m^2 (Darrigran *et al.* 2003) (Fig. 5). A similar trajectory was observed in populations in the Guaiba basin (Brazil) in 1999 (Dreher Mansur *et al.* 2003).

Impact on natural environments

The rapid increase in density and distribution of this invading bivalve added a widespread and abundant epifaunal mussel to freshwater benthic communities of the Plata basin. Dense populations of golden mussels create new habitats that can be colonized by other taxa, thereby modifying the specific richness and composition of the native benthic communities.

Little quantitative information is available regarding the original composition of the benthic freshwater fauna of the

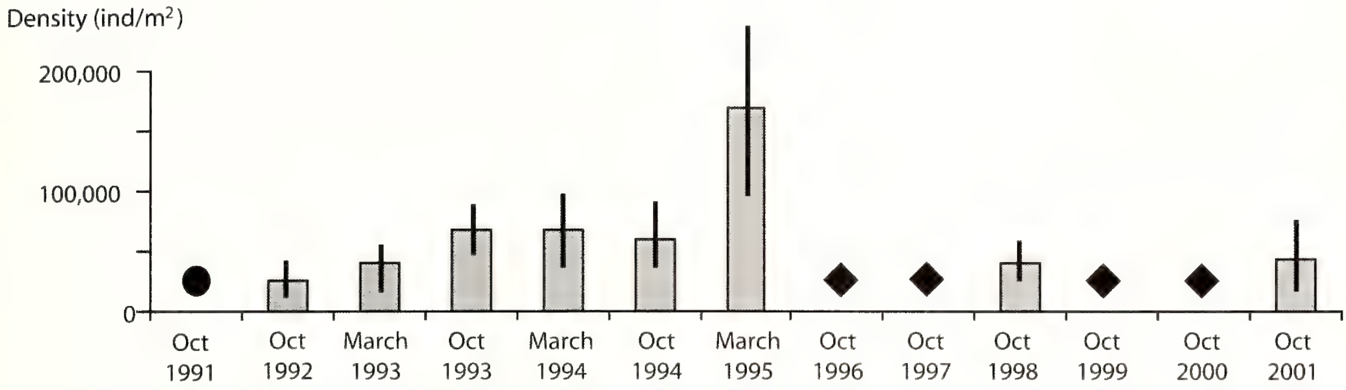

Figure 5. Temporal variation of density (mean and standard deviation) of *Limnoperna fortunei* in Bagliardi Beach, Río de la Plata, between 1991 and 2001. Black circle, 4-5 individuals per m^2; black rhombus, no data (modified from Darrigran *et al.* 2003).

Plata basin. In 1988, at Bagliardi Beach, before the introduction of *Limnoperna fortunei,* three gastropods were common in the rocky environment: *Heleobia piscium* (d'Orbigny, 1835), *Chilina fluminea* (Maton, 1809), and *Gundlachia concentrica* (d'Orbigny, 1835) (Darrigran 1991). Only two species of Hirudinea (Gullo and Darrigran 1991) and one species of Isopoda (Darrigran and Rioja 1988) were found. By 1992-1995, high densities of *L. fortunei* at this location had created a new microenvironment; *C. fluminea* and *G. concentrica* had become rare, while other epifaunal macroinvertebrates were recorded for the first time: eight species of Oligochaeta, one Aphanoneura, eight Hirudinea, and other species of Amphipoda, Tanaidacea, Isopoda, Chironomidae, Turbellaria and Nematoda (Darrigran *et al.* 1998b).

The most direct and severe ecological impact has been the epizoic colonization of native naiads (Hyriidae and Mycetopodidae) by *Limnoperna fortunei,* similar to the impact of *Dreissena polymorpha* on native bivalves in North America (Ricciardi *et al.* 1997). The displacement of the native naiads resulted from their inability to open and shut their valves because of the byssally-attached mussels on their shells. The quantitative impact of *L. fortunei* on native naiads in South America is unknown. Golden mussels also settle on other native fauna, such as *Pomacea canaliculata* (Lamarck, 1822) (Gastropoda, Ampullariidae) and *Aegla platensis* Schmitt, 1942 (Anomura, Aeglidae), as well as on the introduced *Corbicula fluminea* (Bivalvia, Corbiculidae) (Darrigran *et al.* 2000, Darrigran 2002).

The large biomass associated with high densities of *Limnoperna fortunei* impacts aquatic food chains. Several species of native fish consume *L. fortunei* (López Armengol and Casciotta 1998, Montalto *et al.* 1999) and it has become the main food source for *Leporinus obtusidens* (Valenciennes, 1836) (Anostomidea) in the Río de la Plata (Penchaszadeh *et al.* 2000).

Impact on human environment

The introduction of *Limnoperna fortunei* impacts not only natural environments. Freshwater macrofouling, caused by *L. fortunei,* is a novel economic problem in South America. Previously, macrofouling was only a problem in coastal and estuarine localities. Now, however, major industries in Argentina, Brazil, Uruguay, and Paraguay are faced with problems including reduction of water-pipe diameter, blockage of pipelines, decrease of water velocity, accumulation of empty shells, contamination of water by dead mussels, and blockage of filters by larvae and juveniles and their settlement in different parts of the processing plants (Darrigran 2000). These problems have been recorded in numerous installations, including water purifying plants, hydroelectric plants, thermal plants, freezing plants, and oil factories. As a consequence, costs rise because of shutdowns caused by pipeline obstructions and the need for periodic mechanical or chemical cleaning as well as the replacement of pipes and filters. Most information on this issue is contained in technical reports that are not widely available.

The global importance of invasion by *Limnoperna fortunei*

The climate in the native range of *Limnoperna fortunei* is humid subtropical, without a dry season and with a warm summer. Many parts of the world have similar climates, including parts of Japan and the Plata basin, both already invaded by this bivalve. Ricciardi (1998) has alerted us to the possibility of invasion of North America. In particular, the Gulf of Mexico and the Mississippi basin also have a similar climate and are therefore probably susceptible to invasion. Two important ocean navigation routes are also associated with this area: the Atlantic coast, with 30% of the USA traffic, and the Gulf of Mexico, with the major port of New Orleans. Invasion could be in ballast water of ships from southeast Asia or South America. European harbors are also susceptible to invasion.

Research on *Limnoperna fortunei* in South America

Introduction of the golden mussel in the Plata basin has resulted in extensive research activities, including: impacts on native species (Darrigran *et al.* 1998b); individual growth rates (Boltovskoy and Cataldo 1999, Maroñas *et al.* 2003); distribution and impacts (Darrigran 2000, Darrigran *et al.* 2000, Darrigran and Ezcurra de Drago 2000); reproductive biology (Darrigran *et al.* 1998a, 1999, 2003a); predation (Penchaszadeh *et al.* 2000); larval development and larval ecology in natural environments (Cataldo and Boltovskoy 2000; Irurueta *et al.,* in press) and in human environments (Darrigran *et al.* 2003b); resistance to exposure to air under different conditions of relative humidity, aimed at developing low environmental impact control methods and at determining the mussel's capacity for further spread (Darrigran *et al.* 2004); preliminary bioassays to determine the lethal effect of copper and zinc in order to formulate antifouling paints and coatings for freshwater environments (Caprari and Lecot 2001); and bioassays with a polymeric quaternary ammonium, tested on larvae (Darrigran *et al.* 2001) and adults (Darrigran and Damborenea 2001).

Most of these studies of *Limnoperna fortunei* are ongoing and will permit comparison with the abundant information on the invasion of *Dreissena poymorpha* in North America, as they are morphologically and functionally similar species. However, many other aspects of the biology of *L. fortunei* are still unknown, including its filtering capacity. Because of its high density in the Plata basin, *L. fortunei* could increase water clarity in a manner similar to that caused by *D. polymorpha* in North America (Fanslow *et al.* 1995).

The overall aim of the research activities outlined above is to provide the necessary information for effective control of the invasion and for mitigating its economic impact. Likewise, the results obtained should provide valuable information for prediction of future invasions (Ricciardi 2003), the impact this species may have in other regions, and basic information for the design of sustainable strategies of prevention and control.

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Invasion of the clonal clams: *Corbicula* lineages in the New World*

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Abstract: Unknown in the New World prior to 1924, members of the exotic bivalve genus *Corbicula* now ranges from Michigan to Patagonia and are among the most common freshwater molluscs in the Americas. Michigan populations are triploid and share a distinct shell morphotype (Form A), mitochondrial (mt) haplotype, and nuclear ribosomal genotype with New World populations spanning temperate, subtropical, and tropical watersheds over 85° of latitude. Two additional mt and nuclear ribosomal lineages were detected in a minority of New World populations; all three produced biflagellate sperm, a morphological marker for clonality in this clam taxon. Two of 12 New World samples exhibited qualitatively distinct nuclear/organellar genetic disjunctions. One of these (Iguazú Falls) appears to have resulted from hybridization, but the other (Rio Grande Form A) appears inconsistent with hybridization, and we propose that it may have resulted from *in situ* androgenetic clonal capture. These clones of *Corbicula* are but several of hundreds of recent New World freshwater exotics. Their extraordinarily rapid bicontinental spread may well be a portent of things to come. We anticipate that over the next decades many other New World freshwater exotics will gradually attain extensive bicontinental distributions.

Keywords: alien clam, freshwater, androgenesis, phylogeny, genetic disjunction

Continental ecosystems are being profoundly reshaped by ongoing human-mediated transoceanic biotic exchange (Vermeij 1996). Freshwater biotas that were previously insulated by oceanic barriers to dispersal are now vulnerable to inter-continental alien introductions, and a large variety of such invasive taxa have recently become established in New World watersheds (Benson 1999). In North America, much research attention is focused on the Eurasian "zebra mussel," *Dreissena polymorpha* (Pallas, 1771) (Nalepa and Schoesser 1993). The geographic scale of its spread in the New World, however, is dwarfed by that of the "Asian clam" *Corbicula* Mühlfeld, 1811, another freshwater alien.

Individuals of the genus *Corbicula* were first recorded in the New World in western North America almost 80 years ago, and, after breaching the Continental Divide in the late 1950s, members of the genus rapidly extended across the continental USA and into Mexico (McMahon 1999). In South America, the genus has spread extensively in the quarter century since its first detection in southern Brazil and Argentina (Ituarte 1994), becoming established in Venezuela in the 1980s (McMahon 1999) and subsequently in the Panatal (Callil and Mansur 2002), as well as the lower (Beasley and Tagliaro 2001) and upper (this study) sections of the Amazonian drainage system. At present, New World populations of *Corbicula* range from Lake Superior (Ward and Hodgson 1997) to Patagonia (Cazzaniga 1997), dominate the benthic macrofauna of many warm temperate-to-tropical New World watersheds, and frequently impede domestic and industrial water supply infrastructure (Ituarte 1994, McMahon 1999). Our aim in this study was to perform a phylogenetic analysis of this hyper-invasive alien focusing on its current New World distribution, a task complicated by the unusual genetic structure and obscure sister relationships of many freshwater lineages of *Corbicula*.

Background on Old World Species of *Corbicula*

Prior to the global transoceanic spread of freshwater lineages in the 20th century, the extant range of the clam genus *Corbicula* was restricted to Eurasia (excluding Western Europe), Africa, Madagascar, and Australia (Pilsbry and Bequaert 1927). The most contentious issue in the taxonomy of *Corbicula* has traditionally involved the status of hermaphroditic Asian freshwater lineages. They exhibit considerable shell phenotypic variation, at least some of which is ecophenotypic (Kijviriya *et al.* 1991), and Morton (1986) proposed that there exists but a single species of freshwater

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Corbicula. Subsequent research has shown this to be an oversimplification. In addition to distinct gonochoric freshwater species in Japan (Lake Biwa; Hurukawa and Mizumoto 1953), Indonesia (Glaubrecht and Korniushin 2003), and, as an exotic, Western Europe (Rajagopal *et al.* 2000), a variety of hermaphroditic clonal lineages have been uncovered in Asia. These latter lineages lack meiosis, generate unreduced biflagellate sperm cells, and come in diploid (Komaru *et al.* 1997, Komaru and Konishi 1999, Park *et al.* 2000), triploid (Okamoto and Arimoto 1986, Komaru *et al.* 1997, Komaru and Konishi 1999, Park *et al.* 2000, Qiu *et al.* 2001), and tetraploid (Qiu *et al.* 2001) genomic iterations. Remarkably, at least some of these clonal lineages, including diploid and triploid clones, are androgenetic, an unusual method of reproduction in which the embryonic nuclear genome is inherited paternally (derived from an unreduced sperm nucleus) and the maternal nuclear genome is ejected via the polar bodies (Komaru *et al.* 1998, 2000, Ishibashi *et al.* 2002, 2003, McKone and Halpern 2003). Pending identification of convincing sexual parental species, the evolutionary relationships and taxonomic nomenclature of these diverse clones remain obscure (Siripattrawan *et al.* 2000). Identifying parental taxa may be relatively complicated as emerging data suggest that nuclear/mitochondrial disjunctions and introgressive events may be quite common in freshwater lineages of *Corbicula* (Park *et al.* 2002, Pfenninger *et al.* 2002).

Background on Alien New World *Corbicula*

The systematic uncertainty concerning hermaphroditic Asian freshwater *Corbicula* taxa has inevitably complicated the study of exotic New World lineages. Three distinct shell morphotypes have been distinguished. Two morphotypes occur in North America (Hillis and Patton 1982), a widespread "Form A" and a southwestern "Form B" (Britton and Morton 1986) (Fig. 1). A third morphotype, "Form C" (Fig. 1), previously identified as *Corbicula largillerti* (Philippi, 1844), has been described in La Plata, Argentina (Ituarte 1994). All three New World morphotypes are hermaphroditic (McMahon 1991, Ituarte 1994). Attempts to genetically characterize New World populations of *Corbicula* have been restricted to North American temperate populations where the Form A and Form B morphotypes represent two distinct allozymic (Hillis and Patton 1982, McLeod 1986) and mitochondrial (Siripattrawan *et al.* 2000) lineages. Preliminary karyological characterization has been performed on samples of Form A and Form B morphs and both have been found to be triploid ($3n = 54$), to lack meiotic metaphases, and to produce unreduced sperm (Burch *et al.* 1998, J. B. Burch, pers. comm.).

The rapid spread of multiple alien morphotypes and lineages of *Corbicula* across bicontinental New World temperate, subtropical, and tropical watersheds raises obvious questions regarding the genetic diversity associated with this enormous range extension. Our study aimed to provide an initial estimate of genetic structuring in these exotic populations, using both mitochondrial and nuclear markers.

MATERIALS AND METHODS

Sampling

Live animals were sampled at each of the 12 study locations, which collectively spanned the bicontinental New World range. All specimens sampled outside of Michigan were preserved in 95% ethanol prior to shipment. Voucher specimens for each location have been deposited in the University of Michigan's Museum of Zoology Mollusk Division. Details of the sampling site locations, date sampled, collector, UMMZ catalog number, and GenBank Accession numbers are given in Table 1.

Morphotype Designation

Freshwater morphs of *Corbicula* exhibit a significant degree of ecophenotypic variation in shell phenotype, especially in shell outline, shape, sculpture, and pigmentation (Prezant *et al.* 1988, Kijviriya *et al.* 1991, Tsoi *et al.* 1991, Ituarte 1994). Although the two North American morphs (Form A and Form B) have been previously dismissed as ecophenotypes (Britton and Morton 1986), this interpretation is inconsistent with the maintenance of pronounced morphological distinctiveness in microsympatric populations (McLeod 1986, present study) and with the presence of fixed multi-locus differences among the morphs (Hillis and Patton 1982, McLeod 1986, present study). North American Form A is easily distinguished (especially in 3 dimensions) from Form B by its more trigonal shape, taller and more inflated umbos, coarser and more widely spaced external commarginal ribs, and lighter colored inner shell surface (McMahon 1991; Fig. 1). These distinctions are most obvious in juvenile specimens (Hillis and Patton 1982), and they were unambiguously evident in our microsympatric specimens of Form A and Form B from the Rio Grande study site. The Form C morphotype has by far the finest external surface sculpture and the thinnest and least inflated shell of all three New World morphotypes. It is further distinguished from the co-occurring Form A morphotype by the latter's prominent umbo, posterior rostrum, and lighter coloration (Ituarte 1994; Fig. 1).

Cytological Methods

Cytological investigation of clams of *Corbicula* Form A from Michigan was performed using an acetic orcein squash technique (Lee 1999). Freshly sampled clams were injected in their visceral masses with 0.2 ml of 0.05% colchicine. After a 2 hour incubation, the gonads were dissected, hypo-

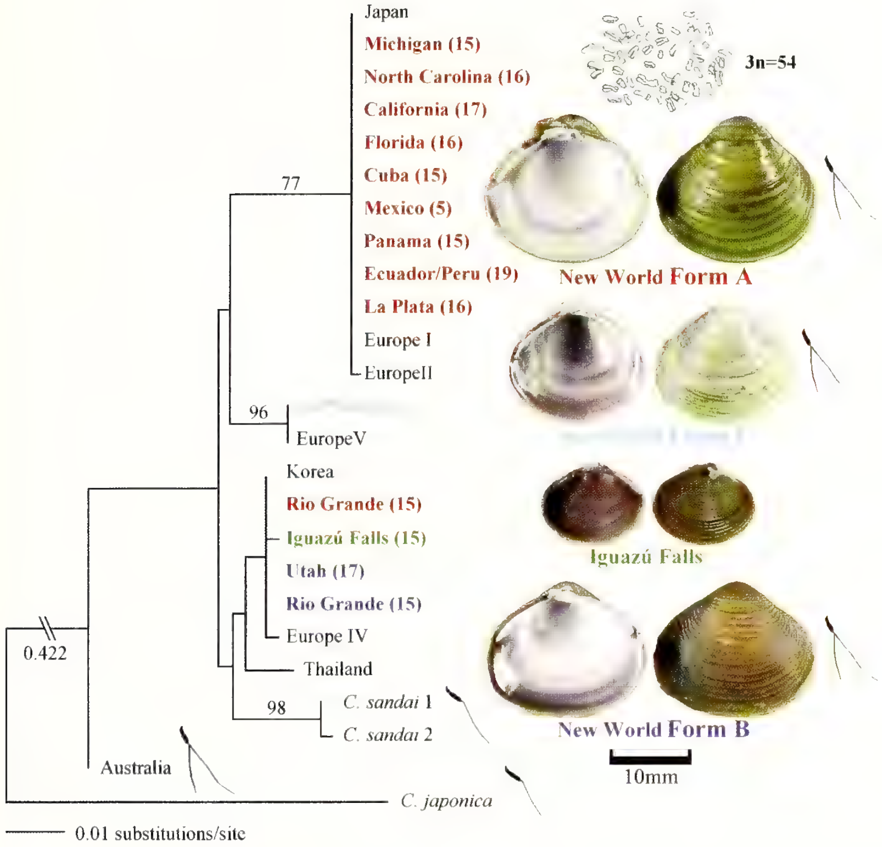

Figure 1. Maximum-likelihood phylogram (lnL = -1171.63) of COI DNA sequences of freshwater individuals of *Corbicula*. The ML tree was recovered with PAUP using the HKY+I+Γ model of nucleotide substitution and rooted with the Asian estuarine sexual species *Corbicula japonica*. The ingroup contained a second Asian diploid sexual species (the Lake Biwa endemic *Corbicula sandai* Reinhardt, 1878). The known sperm morphology of the species/morphotypes of *Corbicula* is indicated by monoflagellate (sexual) and biflagellate (clonal) sperm icons (unknown for the Iguazú Falls morphotype). Bracketed numbers after each New World sampling location indicate the number of individuals bearing that haplotype. Upper numbers on ingroup branches indicate Bayesian posterior probability values > 50. Exemplars of the three primary New World shell morphotypes A, B, and C, and of the Iguazú Falls morphotype are depicted showing internal views of the left valves and external views of the corresponding right valves. New World mt haplotypes recovered from each study population are color-coded to indicate which shell morphotype(s) they were associated with. A triploid (3n = 54) mitotic metaphase chromosome spread is depicted from our Michigan sample of New World Form A.

tonically treated, fixed with Carnoy's solution, stained with 1% acetic-orcein, macerated, squashed on a microscope slide, and viewed with a compound microscope. Metaphase spreads were drawn with the aid of a camera lucida.

Molecular Methods

DNA templates were extracted from mantle edge tissue using a DNeasy Tissue Kit (QIAGEN) according to the manufacturer's protocols. Two target gene fragments were PCR-amplified: a 656 nucleotide (nt) portion of the mt gene *Cytochrome oxidase I* (COI) using primers LCO1490/HCO2198 (Folmer *et al.* 1994) and a 412 nt Domain 2 28S nuclear ribosomal DNA fragment using primers D23F and D4RB (Park and Ó Foighil 2000). The target fragments were amplified with 4 units of *Taq* DNA Polymerase (Promega, storage buffer A) and a negative control (no template) was included in each PCR run. For all PCR reactions, a touchdown protocol (Palumbi 1996) was utilized. After a 3 min 94°C denaturation, an initial annealing temperature of 65°C was decreased by 2°C/cycle (40 s 94°C denaturing, 40 s annealing and 1 min 72°C extension) until the final annealing temperature (45°C for COI and 55°C for 28S) was reached and subsequently maintained for an additional 30 cycles. The resulting double-stranded PCR products were isolated on 1% agarose gels, excised under long wavelength UV light, and purified using a QIAEX II Gel Extraction Kit (QIAGEN). Both strands of the amplified fragments were directly cycle-sequenced using the original amplification primers (annealing temperature 50°C) and Big-Dye Terminator Cycle Sequencing Ready Reaction (Perkin Elmer Applied Biosystems). The sequencing reactions were cleaned using Centri-Sep spin columns (Princeton Separations) loaded with G-50 Sephadex (Sigma) and then electrophoresed on an ABI 377 (Applied Biosystems) automated DNA sequencer.

Phylogenetic methods

Our COI phylogenetic data set for morphs of *Corbicula* incorporated sequences from earlier studies: Siripattrawan *et al.* 2000 (GenBank AF196268–74; AF196278; AF196278; AF196281) and Renard *et al.* 2000 (GenBank AF269090; AF269094–96), in addition to novel New World sequences obtained in this study (GenBank AF519495–519512). In all analyses, the estuarine, diploid, meiotic, dioecious, non-brooding, indirect-developer *Corbicula japonica* Prime, 1864 was utilized as an outgroup (Siripattrawan *et al.* 2000). Phylogenetic analyses were carried out under maximum parsimony (MP) and maximum likelihood (ML) optimality criteria using PAUP*4.0b10 (Swofford 2002). ML searches (heuristic, 5 random sequence additions) were carried out under the HKY model (Hasegawa *et al.* 1985) with rate heterogeneity. The transition/transversion ratio, proportion of invariable sites, and gamma shape parameter were estimated by maximum likelihood. To estimate the phylogenetic uncertainty of the tree topology obtained, we utilized a Bayesian statistical method with Markov chain Monte Carlo sampling (Huelsenbeck *et al.* 2000) using MrBayes 3.0 (Ronquist and Huelsenbeck 2003). We sampled 40,000 trees and discarded the first 8,000 found to ensure the Markov chains had converged on a stationary posterior probability distribution. A 50% majority-rule consensus was obtained from the remaining trees and the proportion of trees containing a specific branching order represented its posterior probability given the data and model of evolution. Unweighted MP analyses yielded 5 most parsimonious trees (92 steps, CI = 0.8152, RI = 0.9000) in which the Australian and Thai mt lineages had alternate topological placements within the ingroup crown clade.

RESULTS

Morphotype distribution

Eleven of the 12 sampling locations yielded clams that could be readily assigned to one of the three New World morphotypes of *Corbicula* (Table 1; Fig. 1). Form A was by far the most common and was encountered in 10/12 bicontinental locations spanning the New World range of the genus *Corbicula* (Table 1; Fig. 2). Typically, Form A was the only morphotype present at these locations. However, in two cases it occurred in microsympatry: in the Rio Grande with Form B and in La Plata with Form C (Table 1). In addition to its presence in the Rio Grande, the Form B morphotype was also encountered (on its own) in a Utah watershed (Table 1). The Iguazú Falls sample was an exception to the three morphotype system. Clams from Iguazú Falls, although most closely resembling Form C, were noticeably less trigonal and had slightly coarser and more widely-spaced external commarginal ribs (Fig. 1).

Mitochondrial Haplotype Distribution

A total of 212 individuals, retrieved from 12 sampling locations spanning the bicontinental New World range (Table 1), were sequenced for the target COI gene fragment. Eleven of the 12 study populations were monotypic, *i.e.*, only one mt genotype was encountered within each population. A total of four New World haplotypes were recovered comprising three phylogenetically distinct mitochondrial lineages, all of which nested firmly within a shallow crown clade of predominantly clonal Austral/Asian freshwater congeners (Fig. 1).

Our New World samples were dominated (150/212 individuals) by a single mt haplotype which was present in nine temperate, subtropical, and tropical sampling locations and was exclusive to the Form A morphotype (Fig. 1). Remarkably, its distribution encompassed the New World latitudinal range of the genus *Corbicula* (Fig. 2). Outside of the New World, the Form A mt haplotype occurs widely in Asia (Park and Kim 2003) and has been recovered from an androgenetic Japanese triploid (3n=54) clone (Komaru *et al.* 1998, Siripattrawan *et al.* 2000; Fig. 1). Karyological examination of the Michigan study population of Form A showed that it too was triploid (3n=54; Fig. 1). The Form A mt haplotype has also been recovered in European exotic populations (Renard *et al.* 2000, Fig. 1), where they occur in diploid individuals (Pfenninger *et al.* 2002).

A second mitochondrial lineage, previously isolated from the North American Form B shell morphotype (Siripattrawan *et al.* 2000), also had a bicontinental distribution. Two haplotypes were encountered, one in populations from western interior USA (Rio Grande, Utah), the other, which differed by a single synonymous substitution, in the Iguazú Falls section of the Paraná drainage system (Figs. 1, 2). Both of our New World samples of the Form B morphotype, from the Utah and Rio Grande populations, were fixed for the former haplotype. However, unlike the other two New World mt genotypes, the Form B mt lineage was not exclusive to a single New World morphotype. In the Rio Grande, where Form A and Form B morphotypes were sampled in microsympatry, the 15 Form A individuals typed also had the North American Form B mt haplotype (Fig. 1). In addition, all 15 of the typed clams from Iguazú Falls had the South American Form B mt haplotype in association with a distinctive shell morphotype that most closely resembled La Plata Form C (Fig. 1). Outside of the New World, the North American haplotype is widespread in Asia (Park and Kim 2003) and has been recovered from a triploid (3n=54) Korean clone (Siripattrawan *et al.* 2000, Park *et al.* 2000). A minor variant of this mt lineage is also present in exotic Western European populations (Renard *et al.* 2000; Fig. 1).

The third New World mt lineage was represented by a single haplotype found in clams with the morphotype Form C shell from the La Plata region of Argentina (Figs. 1, 2). Although Form A and Form C morphotypes from La Plata were sampled in sympatry (*i.e.*, the same stream), they were fixed for distinct mt genotypes (Fig. 1). The Form C haplotype was phylogenetically distinct from the available repertoire of Austral/Asian mt diversity of freshwater species of *Corbicula*, although it also occurred in exotic Western European populations (Renard *et al.* 2000, Fig. 1).

Nuclear genotype distribution

We cross-profiled a sub-sample (89 individuals) of our

Table 1. Sampling particulars for New World specimens of *Corbicula,* together with UMMZ catalog numbers and GenBank Accession numbers.

Population	Locality	Morphotype/ # sequenced for COI	Date	Collector	UMMZ#	GenBank# (COI/28S)
Michigan	Davis Creek, South Lyon, Michigan, USA. 42.462°N, 83.708°W.	Form A 15	September 10, 1999	R. S. Mulcrone	300014	AF519495 AF519513
Utah	Small stream, 1 mi. south of southern exit to Brigham City along interstate 15, Box Elder County, Utah, USA. 41.482°N, 112.062°W.	Form B 17	November 1, 1999	P. and M. Hovingh	266695	AF519509 AF519528
California	SFPUC Station 12, Upper Crystal Springs Reservoir, San Mateo County, California, USA. 37.509°N, 122.35°W.	Form A 17	August 15, 2000	J. Bielski	300015	AF519497 AF519515
North Carolina	Little Fishing Creek (Tar River Basin), 1 mi. west of White Oak, Halifax County, North Carolina, USA. 36°11′09″N, 77°52′36″W.	Form A 16	July 7, 1998	A. Bogan	266694	AF519496 AF519514
New Mexico	Rio Grande at Mesilla Bridge, Las Cruces, New Mexico, USA. 32.291°N, 106.826°W.	Form A 15 Form B 15	December 4, 2001	D. Ó Foighil	300016(A) 300017(B)	AF519510(A) AF519526(A) AF519511(B) AF519529(B)
Florida	Santa Fe River at High Springs, Alachua County, Florida, USA. 29.827°N, 82.597°W.	Form A 16	May 2001	P. Baker	300018	AF519498 AF519516
Cuba	Parque Lenin, Havana, Cuba. 23.102°N, 82.381°W.	Form A 15	January 3, 2002	A. Gutierrez	300019	AF519499 AF519517
Mexico	3 locations in Jalisco, Mexico: Balneiro de Teuchitlan, El Arco, Rancho La Huerta, Buenos Aires.	Form A 2 1 2	May 10-11, 2000	S. Webb	300020 300021 300022	AF519500 AF519518 AF519501 AF519519 AF519502 AF519520
Panama	Panama Canal System at Gamboa Bridge, Panama. 9°6′57″N, 79°41′49″W.	Form A 15	April 27, 2002	D. Ó Foighil	300023	AF519503 AF519521
Ecuador	Río Pastaza at Consuelo, Ecuador. 1°55′9″S, 77°48′52″W.	Form A 15	July 18, 1999	J. Sparks	300024	AF519504 AF519522
Ecuador	Río Pastaza at Warany, Ecuador. 2°14′9″S, 77°15′10″W.	Form A 3	July 16-17,1999	J. Sparks	300025	AF519505 AF519523
Peru	Río Pastaza at Laguna Rimachi near Cano Rimachi, Peru. 04°25′8″S, 76°40′33″W.	Form A 1	August 14, 1999	J. Sparks	300026	AF519506 AF519524
Iguazú Falls	Isla San Martín, Iguazú Falls, Misiones Province, Argentina. 25°41′39″S, 54°25′36″W.	Iguazú Falls 15	November 8, 1995	C. F. Ituarte	300027	AF519512 AF519530
Argentina	Unnamed brook, 17 km south of La Plata City, Buenos Aires, Argentina. 34°57′37″S, 57°46′37″W.	Form A 16 Form C 16	February 18 and May 28, 2000	C. F. Ituarte	300028(A) 300029(C)	AF519507(A) AF519525(A) AF519508(C) AF519527(C)

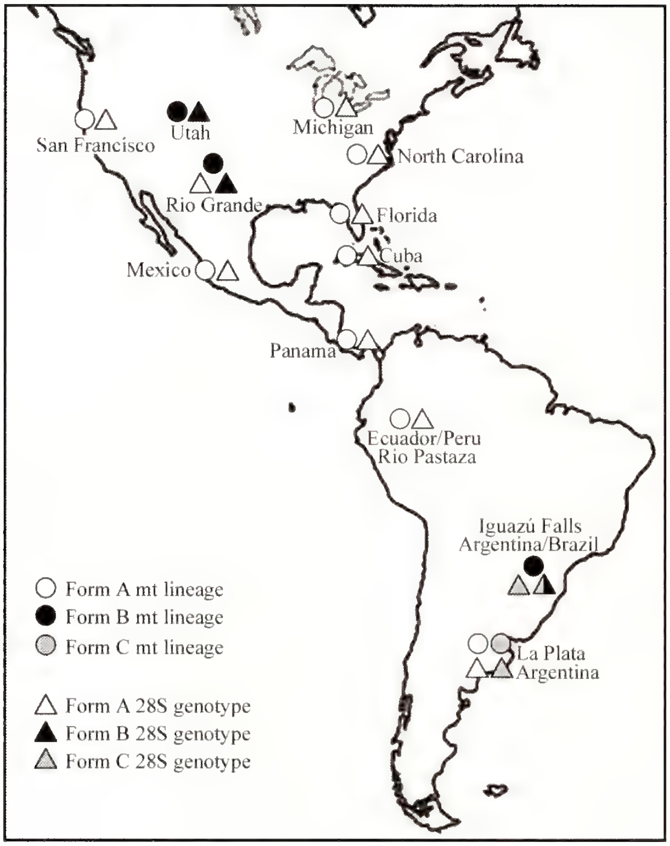

Figure 2. Summary map showing the distribution patterns of the three mt lineages and the three 28S nuclear genotypes detected among our 12 New World sampling sites of *Corbicula*.

study populations for nucleotide variation in a 412 nt segment of the large subunit (28S, Domain 2) nuclear ribosomal gene. Three 28S genotypes were detected in New World specimens of *Corbicula* (Fig. 3), and there was near-perfect congruence among shell morphotype (Fig. 1) and nuclear 28S genotype. The exception was a minority (4/15) of Iguazú Falls morphotypes that displayed heterozygous chromatograph profiles containing both Form C and Form B 28S genotypes (Fig. 2). Significantly, individuals from 9 bicontinental populations bearing the Form A mt haplotype and shell morphotype (Fig. 1) exclusively exhibited the Form A 28S genotype (Fig. 2).

We encountered evidence of nuclear/organellar genetic disjunction in two New World samples. All 15 clams of Form A shell morphotype from Rio Grande investigated had a Form A nuclear 28S genotype and a Form B mt COI genotype (Fig. 2). Correspondingly, all 15 clams of Form C shell morphotype from Iguazú Falls investigated had a Form C nuclear 28S genotype (four were also heterozygous for Form B 28S) and a Form B mt COI genotype (Fig. 2).

DISCUSSION

Our results indicate that New World populations of the invasive freshwater clam genus *Corbicula* are composed of a small number of lineages, one of which appears to be predominant in temperate, subtropical, and tropical ecosystems. Remarkably, the triploid Form A lineage present in the Huron River watershed in Ann Arbor, Michigan, shared the same COI haplotype and 28S genotype with multiple bicontinental populations of Form A, including those in such geographically and ecologically diverse locations as Cuba, the Panama Canal system, the headwaters of the Amazon, and La Plata, Argentina (Figs. 1-2). Although our preliminary results require testing with more rapidly evolving nuclear markers, they are consistent with the surprising hypothesis that the vast New World range expansion of the genus *Corbicula* may be predominantly composed of the Form A lineage, which appears to have a realized broad-spectrum ecophysiological scope.

The limited data available on reproductive mode(s) of alien New World morphs of *Corbicula* are consistent with clonality, especially for studied North American Form A and Form B populations that are triploid (3n = 54; confirmed for Michigan Form A in this study), lack meiotic metaphases, and produce unreduced sperm (Burch *et al.* 1998, J. B. Burch, pers. comm.). Morphs of Form A also occur in Central and South America, and share the same mt and nuclear genotypes with triploid North American samples (Fig. 2), but their ploidy status remains to be determined. However, La Plata populations of both Form A and Form C produce biflagellate sperm (C.F. Ituarte, pers. obs.), as do North American populations of Form A and Form B (Kraemer 1983, Siripattrawan *et al.* 2000). This trait has been proposed as a morphological marker for clonality in lineages of *Corbicula* (Siripattrawan *et al.* 2000). The rationale for this hypothesized linkage is two-fold. Biflagellate sperm are extremely rare in the Mollusca and have only been recorded, apart from occasional aberrant individual cells (J. M. Healy, pers. comm.), from hermaphroditic, freshwater lineages of *Corbicula*. Quantitative DNA analyses of biflagellate sperm from Asian 2n (Komaru *et al.* 1997, Ishibasi *et al.* 2003), Asian 3n (Komaru *et al.* 1997), Asian 4n (Qiu *et al.* 2001), hermaphroditic, freshwater lineages of *Corbicula,* and also from 3n North American exotics (Burch *et al.* 1998), have consistently found them (unlike their sexual monoflagellate congeners [Konishi *et al.* 1998]) to be unreduced, having the equivalent DNA content to somatic cells. Loss of meiosis is thought to have originated in clonal lineages of *Corbicula* by abortive cytokinesis of the 1st or 2nd meiotic divisions (Komaru *et al.* 1997). A disjunction between the various signalling pathways that control distinct aspects of cellular replication could conceivably result in a situation where some

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        1                                                                                                    104
Form A  AACCGCATAGAGCCAAACGGGTGGATCCGCAGAGTCGACCCGGGGAATTCAGCCCGGCCGCCAGCGGCGCTCGCAAAGTTCGGATCCGCAAGGACCGGGCCAGC
Form B  .............................................................A..........................................
Form C  ..............................................................CA...A....................................

        105                                                                                                  208
Form A  GGGACGTCCGCGTGGCAGGGTGCACTTTCTCCGCGGTCGAGTGCTACGACCGGTTTCGAGGCGGTCAGAAGCCCGGCGGAAAGGTAGCGCCGCCCTTCGGGGTG
Form B  ........CA..............................................................................................
Form C  ..........................................................C....................................C........
        209                                                                                                  312
Form A  TCGTGTTATAGGCCGCCGCGGTGGACTCGCCGCGAGACCGAGGACGCTTGCCGCGCCGAGCGGTCCCGGGCCGTCTTGGGAGGTTCGACCTCGGCTGCACTGCT
Form B  ........................................................................................................
Form C  ........................................................................................................

        313                                                                                              412
Form A  CCGCAGAGCACCGTAACCGCCGCCCTAGCACGGGTCCGGAGACTGCCGGCGCCTCGGGTCAGTAGCGAATCGGTCGGTCCTCCACCCGACCCGTCTTGAA (51)
Form B  .................................................................................................... (19)
Form C  .................................................................................................... (19)
```

Figure 3. Aligned Domain 2 genotypes of the large subunit (28S) nuclear ribosomal gene obtained from a sub-sampling of the 12 New World study populations of *Corbicula* previously typed for mt COI. A total of 89 individuals were characterised for 28S. These included all specimens from samples displaying mt/shell morphotype disjunction (30 Rio Grande and 15 Iguazú Falls specimens) plus a subsample of four individuals/morphotype/location from the remaining 10 New World study locations (GenBank AF519513-519530). Three morphotype-associated genotypes were recovered (see prefixes) and the bracketed number at the end of each sequence indicates the number of individuals encountered in our morphotype subsample bearing that genotype. Dots indicate nucleotide identity to the first sequence presented (Form A) and inferred nucleotide substitutions relative to Form A are shown.

elements of cell division (*e.g.,* nuclear division, formation of a cleavage furrow) were suppressed, whereas others (*e.g.,* centriolar replication, flagellar formation) proceeded independently. The linkage of biflagellate sperm with clonal reproduction is robust for the North American populations of Forms A, B that are known to be triploid and ameiotic (Burch *et al.* 1998, J. B. Burch, pers. comm.). It is more speculative for the South American Form C, which has yet to be karyotyped or to have the DNA content of its sperm quantified.

Clonal animal lineages are typically of hybrid origin and the introgressive events leading to their genesis may result in the uncoupling of organellar and nuclear genome genealogies (Kraus and Miyamoto 1990). We encountered evidence of qualitatively distinct nuclear/organellar genetic disjunction in two New World samples: Rio Grande Form A and the Iguazú Falls specimens (Fig. 2). In the former case, the disjunction was complete: all 15 Rio Grande Form A specimens typed had Form B mitochondria in a Form A nuclear setting (Form A 28S genotype, Form A shell morphotype). It was more nuanced in the latter case: all 15 Iguazú Falls specimens typed had Form B mitochondria in a heterogeneous nuclear setting (all had the Form C nuclear 28S genotype, four were also heterozygous for Form B 28S; shell phenotype was ambiguous). These two nuclear/mitochondrial disjunct populations might represent independent alien introductions. Another possibility, however, is that they are products of post-introduction interaction among New World clones. The latter scenario is more plausible for the Rio Grande population given that it is our sole sample of sympatric Form A and Form B lineages and also of this particular genetic disjunction.

Parallel nuclear/mitochondrial disjunctions have also been discovered in recent studies of Korean (Park *et al.* 2002) and European (Pfenninger *et al.* 2002) freshwater lineages of *Corbicula.* Both of these studies concluded that the results were consistent with hybridization events. Hybridization is a reasonable explanation for the disjunction at Iguazú Falls due to the detection of heterozygous nuclear genotypes and the formation of a shell morphotype with putatively intermediate characteristics. The absence of the latter two features from Rio Grande Form A specimens is inconsistent with a nuclear genomic hybrid origin in this particular nuclear/mitochondrial disjunction and raises obvious difficulties given that the distribution data indicate that this condition may well have arisen *in situ.* One intriguing possibility is that the Rio Grande disjunction resulted from androgenetic capture of Form B eggs by unreduced Form A sperm in this watershed, *i.e.,* replacement of the Form B maternal nuclear genome by the Form A paternal genome. Androgenesis has been demonstrated in diploid and triploid Japanese clonal lineages of this genus (Komaru *et al.* 1998, 2000, Ishibashi *et al.* 2002, 2003) and may be widespread among freshwater hermaphroditic congeners. Inter-clone androgenetic egg capture would be confirmed by detecting embryos or larvae in broods of Rio Grande Form B bearing exclusively Form A nuclear genomes. Irrespective of the generating mechanism(s), the presence of multiple nuclear/mitochondrial disjunctions in freshwater lineages of *Corbicula* clearly signals that systematic interpretations based solely on mitochondrial lineages (Renard *et al.* 2000, Siripattrawan *et al.* 2000, Glaubrecht and Korniushin 2003, Park and Kim 2003) could be seriously misleading. Our data, although indirect, imply that genetic interaction among clones of *Corbicula* may be mechanistically diverse.

Our results provide a phylogenetic perspective to New World populations of the genus *Corbicula*, and have implications for ecological studies of these prominent freshwater exotics and for their projected evolutionary persistence. Although our data caution against a blanket application of ecophysiological datasets to all New World populations, they facilitate meaningful comparative studies of the geographically widespread Form A lineage in bicontinental temperate, subtropical, and tropical freshwater ecosystems. Form A's presence in remote, ostensibly pristine, habitats, *e.g.*, the Río Pastaza section of the Amazonian drainage headwaters (Table 1, Fig. 2), is an indirect measure of its pronounced invasiveness. In 1999, Conservation International surveyed the aquatic diversity of a 380 km linear stretch of the Pastaza, more than 3200 km upstream from the mouth of the Amazon, and kindly forwarded us their samples of bivalve molluscs. Form A of *Corbicula* represented, by far, the numerically dominant bivalve recovered in both Andean (Ecuador) and lowland rainforest (Peruvian) Pastaza segments. The extraordinarily rapid spread of exotic lineages of *Corbicula* has been attributed to a combination of passive downstream transport via mucous drogue lines (Prezant and Charlermwat 1984, McMahon 1999), natural spread by phoresy among adjacent watersheds, and human-mediated introductions to geographically disjunct drainages. The putative clonal nature of New World lineages of *Corbicula* may act to enhance further their invasive prowess because single, distantly translocated, juvenile clams could readily found entire new populations.

There are of course potential downsides to obligate clonality. According to the Red Queen hypothesis, the evolutionary trajectory of New World clonal lineages of *Corbicula* will be truncated once the endemic parasitic biota adapts to their ecological presence (Lively and Dybdahl 2000). In this scenario, long-term evolutionary persistence of clones of *Corbicula* in the New World will depend on the generation of genetic novelty through either clonal introgression and/or the continued introduction of fresh exotic clones. The latter process may already be playing out in the La Plata region of Argentina where the formerly abundant Form C is being replaced by the now dominant Form A clone (Darrigran 1991).

The speed of the spread of *Corbicula* lineages across bicontinental New World watersheds is such as to invite its dismissal as an aberration that has little generality. Unfortunately, it may instead be a portent of things to come, and we anticipate that over the next decades a significant fraction of the hundreds of freshwater New World invasive taxa (Benson 1999) will gradually attain extensive bicontinental distributions.

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The potential of zebra mussels as a model for invasion ecology*

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Abstract: *Dreissena polymorpha* is one of the most aggressive aquatic invasive species in the northern hemisphere. Its ability to tolerate both brackish and totally fresh waters and to attach to hard substrates, along with its long-lived larval dispersal phase make it unusual among freshwater bivalves and contribute to its success as an invader. Because zebra mussels cause enormous economic damage, there has been extensive research on this species in a wide range of habitat types, providing the opportunity to examine many of the steps in the invasion process. We know that most invasions fail, and that there are many potential bottlenecks each invader must pass through for an invasion to be successful. Here, I outline the steps necessary for successful invasion by zebra mussels in four different types of aquatic habitats: lakes with major shipping ports, isolated inland lakes, lakes connected by surface waters, and river systems. For each step I suggest the life stage most likely to be important for successful invasion and potential control mechanisms that could be used to stop or slow an invasion.

Key words: *Dreissena polymorpha*, zebra mussels, invasive species, aquatic ecology

The zebra mussel, *Dreissena polymorpha* (Pallas, 1771), is one of the most aggressive invasive aquatic species in the northern hemisphere. Native to the brackish waters of the Caspian and Black Seas, the zebra mussel is unusual among bivalves in its ability to tolerate both brackish and totally fresh waters. Given its broad physiological tolerance, this species spread rapidly across Europe over 200 years ago when shipping canals were built to connect the Caspian and Black Seas with the Baltic Sea (reviewed by Karatayev *et al.* 2003).

After this rapid spread across Europe, surprisingly, zebra mussels did not spread to North America until the 1980s. Once discovered in the major ports of the Laurentian Great Lakes, zebra mussels rapidly spread throughout most of the Great Lakes and major connected shipping conduits, including the St. Laurence and Mississippi Rivers, within a few years (Johnson and Carlton 1996, Allen and Ramcharan 2001, reviewed by Karatayev *et al.* 2003). However, zebra mussels have been much slower to colonize isolated inland lakes and waterways. The first colonization of inland, unconnected lakes of North America lagged several years behind the invasion of the Great Lakes (Johnson and Padilla 1996, Padilla *et al.* 1996, Buchan and Padilla 1999). Although they have invaded lakes in 15 states of the U.S., the vast majority of freshwater lakes are not yet invaded, even those in close geographic proximity to zebra mussel sources. As of 2004, they are found in fewer than 370 North American lakes, mainly in the upper mid-west, primarily in the state of Michigan (USGS 2004), and occupy fewer than 1% of suitable lakes (Ramcharan *et al.* 1992a, Koutnik and Padilla 1994) in the regions where they are found. This pattern is consistent with findings in Belarus (former Soviet Union), where, although zebra mussels have been invading for over 200 years, only 17% of over 500 studied lakes were invaded (Karatayev *et al.* 2003).

Zebra mussels are unusual among freshwater animals in general, and differ especially from most freshwater bivalves in their lifestyle and life history. Although they are not closely related to marine mussels, they have many ecological features that are convergent with marine mussels. Rather than burrowing into or settling on top of soft substrates like most freshwater bivalves, they attach to hard substrates like rocks, pieces of shell, and other bivalves, by use of their byssal threads. In general, freshwater systems have few macro-fouling organisms. Therefore, zebra mussels need not compete with other species for attachment sites. Zebra mussels are also unusual among freshwater molluscs in having a life history with a long-lived feeding veliger stage. The length of time larvae are in the plankton depends on local conditions including water temperature and food availability (Sprung 1987, Stoeckel *et al.* 2004a). Larvae drift in the plankton and are moved by water currents that carry them (Schneider *et al.* 2003, Stoeckel *et al.* 2004b). Like small marine bivalves they can also disperse as juveniles and small adults by crawling or drifting (Baker and Mann 1997).

The zebra mussel has gained immense popular attention because it has a large impact on human populations, especially by attaching to human-made constructions such as power plant turbines, intake pipes and turbines for municipal water plants, docks, locks, and ships. Recently, the total cost associated with zebra mussel invasion in the U.S. has been estimated as over $100 million annually (Pimentel *et al.* 2000, 2001), although it is difficult to estimate this figure accurately.

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In addition to direct impacts on human constructions, zebra mussels also have large impacts on natural systems (reviewed by Karatayev *et al.* 1997, 2002). They can attach to many native species, including aquatic plants; slow moving benthic species like unionid bivalves, many of which are endangered in North America; as well as mobile species such as crayfish, which have a hard exoskeleton (reviewed by Karatayev *et al.* 1997, Burlakova *et al.* 2000). They also can have large impacts as ecosystem engineers (reviewed by Karatayev *et al.* 1998, 2002). They are extremely efficient suspension feeders and can clear the water they filter of almost all particles from 0.4 µm to >40 µm, which includes bacteria, cyanophytes, and microalgae, as well as other suspended organic and inorganic matter (Roditi *et al.* 1996, reviewed by Karatayev *et al.* 1997).

Although zebra mussels have been extremely successful invaders, we know that the invasion process in general is rarely successful. The invasion process can be thought of as a series of conditional probabilities; a chain of events must all happen for a species to be successfully introduced and become an important invader in a new habitat; and at different stages of the invasion process, different life stages of the invader are likely to be important. A successful invasion is dependent on the probability that: (1) an individual that is capable of reproducing is picked up by a suitable vector from a source population; (2) the transport vector carries that individual to a suitable habitat; (3) the individual survives transport; (4) the individual (or its offspring if it reproduces during transport) is deposited in the new location and survives until reproduction; and (5) the individual successfully reproduces in the new location, and it or its offspring has continued successful reproduction, recruitment and survival to establish a sustaining population in this new location (Hewett and Hayes 2002).

Thus, the very low rate of success of invasion (the percent of all potential introductions that actually results in a growing population of a new species in a location) is extremely difficult to estimate. Williamson (1996) suggested a rule of thumb that has been termed the tens rule: a 10% success rate for each step in an invasion. However, this "rule" is not based on real estimates, which will undoubtedly be both system- and species-specific, and may depend on certain types of life histories (Kolar and Lodge 2002). Also, the probability of invasion success may differ among different life history stages of the same invader in different habitat types.

Zebra mussels may offer one of the best aquatic examples that will allow us to begin to quantify the invasion process, especially among different habitat types. There has been extensive research on many aspects of the ecology and invasion of zebra mussels, and future research provides the opportunity to examine each of the steps in the invasion process. Because this invader has invaded multiple geographic areas and continues to spread, we may be able to test hypotheses and predictions directly.

Here I outline the steps necessary for successful invasion by zebra mussels, and for each of these steps I focus on four different types of aquatic habitats: lakes with major shipping ports, isolated inland lakes, lakes connected by surface waters, and river systems. For each I suggest which life stage is most likely to be the successful step in the invasion (benthic adults or planktonic veliger larvae), and potential control mechanisms that could be used to stop or slow an invasion. This is not a comprehensive review; most of the enormous literature can be accessed via the major recent reviews I cite. Rather, I attempt to outline briefly the potential of *Dreissena polymorpha* for developing invasion models, try to identify the key life history stages important for the control of invasion, and thus suggest data that will be needed to develop robust models of invasion risk and success, especially for readers less familiar with this literature.

STEPS TO INVASION

Step 1. Reproductively capable individuals are picked up by a suitable vector from a source population

Transported individuals must be capable of reproducing in the environment to which they are transported in order for an invasion to be successful. For species such as the zebra mussel that have separate sexes and are free spawners, in theory all individuals are capable of reproducing, provided they survive to reproductive age and there are compatible individuals of the opposite sex at the final destination that are in close enough proximity for effective fertilization (Levitan and Petersen 1995). At present, however, we do not know the number of individuals or the density of individuals necessary for an initial introduction of zebra mussels to have effective fertilization. As with marine systems (Levitan and Peterson 1995) we would expect water motion, especially currents, to have an impact on fertilization success in these free-spawning bivalves.

Lakes with major shipping ports.—Shipping, including the deposit of ballast water and sediments, is widely recognized as the major vector for the transport of aquatic invaders among ports (Locke *et al.* 1993, Mills *et al.* 1993, Wonham *et al.* 2001, Minchin and Gollasch 2002, Gollasch *et al.* 2002, Grigorovitch *et al.* 2003). The life stage most likely to be picked up in ballast water is the larval stage, rather than the benthic adults. Ships that carry ballast may not stay in port long enough to develop populations of attached adults, and because of the added fuel costs of fouled hulls, ships are generally maintained such that they do not develop large communities of macro invertebrates, especially mussels, although it is possible that mussels may attach to anchors and thus be transported on the outsides of ships (D. Minchin, personal communication). Control would depend on removing or preventing larval stages from being taken up with ballast water or through exchange of ballast water (Minchin and Gollach 2002). The International Convention for the Control and Management of Ships Ballast Water and Sediments was adopted in February 2004 with the aim of controlling introductions through ballast water. Mid-ocean exchange of ballast water is the most widely used method of reducing the potential for transport of invaders; however, mid-ocean exchange usually does not involve a complete exchange of water, and does not remove invaders in ballast tank sediments or attached to tank walls (Wonham *et al.* 2000). In addition, mid-ocean ballast exchange can be unsafe, especially during periods of rough water or storms. Other methods to control unwanted introductions include filtration or sterilization, but each of these methods has its problems and limitations. Many of these methods are difficult to scale up to the water volumes needed to fill ballast tanks, or are so costly as to be prohibitive. Chemical treatments and biocides have unwanted toxicity to non-target species and thus pose serious threats to local species and environments when treated ballast water is released (Waite 2002). The use of deoxygenation of ballast tanks with nitrogen holds promise; although this method is expensive, the shipper's costs are offset by the reduction of ballast tank corrosion (Tamburri *et al.* 2002).

Isolated inland lakes.—In contrast to shipping vessels, personal recreational boats may be in the water at a particular location for weeks to months, allowing the development of adult zebra mussels (Johnson and Carlton 1996, Padilla *et al.* 1996, Buchan and Padilla 1999, Johnson *et al.* 2001). These boats usually do not trap large volumes of water that would contain veligers, but some do have small volume live wells.

Most recreational boats are transported among lakes on trailers. Trailers can snag water weeds, which can have extremely high densities of zebra mussels (Johnson *et al.* 2001). Thus, juvenile and adult zebra mussels are more likely than veliger larvae to be the life stages transported from isolated inland lakes (Johnson *et al.* 2001). Boat anchors could also snag water weeds or be sites for attachment, and thus transport of zebra mussels. Control would depend on removal of attached mussels from the surface of boats, anchors and, more important, removal of all attached or snagged water weeds from trailers that leave a body of water.

Surface water connected lakes.—Although some boat traffic may occur between lakes connected by water, most lakes are connected by small streams or rivers without boat traffic. In these cases, the flowing water among lakes would be the most likely transport vector for dispersing zebra mussels. Although juvenile and adult mussels do move to a certain extent (Baker and Mann 1997), the larval stage is the dominant phase that is transported to new bodies of water.

Control, preventing larvae from traveling with moving water, would be very difficult in water-connected systems. It would require that all veligers be removed from flowing surface waters. Because many other important species are found in flowing waters, selective removal of harmful or pest species, especially those the size of veligers, may be extremely difficult if not impossible in many circumstances.

River systems.—As with surface water-connected lakes, transport of zebra mussels within river systems will be primarily by downstream drift of veligers. However, movement among disconnected river systems will involve the same transport vectors as among isolated lake systems—recreational boats—and if the river system is used for shipping, then the importance of veliger transport though ballast or attachment of adults to barges or other large vessels that remain in the waterway for extended periods of time would also be important. As in surface water-connected lakes, control of veliger transport would be very difficult in river systems. Prevention of ship or barge transport of adults could be managed through requirements that vessels maintain clean hulls and do not move into uninvaded waters, including upstream areas of the same system. If barges hold ballast water, these waters would also need to be cleared of zebra mussels.

Step 2. The transport vector must carry the individuals to a suitable habitat

The transport vector must reliably move to habitats that are suitable for the survival of individuals and the establishment of new populations. For aquatic animals, movement to different bodies of water is essential. Those waterbodies must have the chemistry, food availability, and temporal stability needed by the invader. Ramcharan *et al.* (1992a) found that suitability of lake habitats for zebra mussels depended strongly on water chemistry, especially calcium concentration and pH. Allen and Ramcharan (2001) used the characteristics of invaded and uninvaded river systems to make similar predictions for flowing water. Karatayev *et al.* (1998) reviewed other physical factors that are important in determining suitability of habitats for zebra mussels, including substrate type, oxygen availability, and temperature extremes. The physiological limits of zebra mussels will also be important in their ability to invade habitats (McMahon 1996).

Lakes with major shipping ports.—If a ship visits both freshwater and marine ports, then the likelihood of transporting invaders will depend on the sequence of visitations. If a ship regularly goes to a marine port after a freshwater port or vice versa, then there will be an extremely low probability (or none at all) of invasion. Similarly, if freshwater ballast is exchanged in mid-ocean, depositing all freshwater propagules into sea water, the transport of zebra mussels and other freshwater invaders could be controlled (Wonham *et al.* 2001). Ballast water exchange has been recommended as a means of controlling the spread of both freshwater and marine invaders; however, regulations have been difficult to enforce and have not been totally effective (Wonham *et al.* 2001, Waite 2002).

Isolated inland lakes.—Recreational boats are the most important vector moving zebra mussels among freshwater lakes, and the patterns of boater movements among lakes can be analyzed to determine the risk of spread (Padilla *et al.* 1996, Buchan and Padilla 1999, Johnson *et al.* 2001). If the pattern of lake use by boaters is such that lakes unsuitable for zebra mussels are visited immediately after visiting lakes with zebra mussels, then boater travel will be unlikely to spread zebra mussels. However, if boaters regularly travel from lakes with large populations of zebra mussels to lakes that are suitable for zebra mussels, the probability of invasion and spread increases (Buchan and Padilla 1999).

To control this aspect of invasion, cleaning and removing zebra mussels from all areas outside of boats and trailers would be effective. In addition, efforts to identify candidate lakes that are uninvaded but suitable for invasion, and that are visited by many boaters from lakes with zebra mussels, will be critical to stopping or slowing the spread of zebra mussels. The use of boater surveys coupled with the known presence of populations of zebra mussels and the suitability of bodies of water to support zebra mussels has been used in this way for Wisconsin waters (Padilla *et al.* 1996, Buchan and Padilla 1999), other midwestern states (Johnson *et al.* 2001), and is presently being used to attempt to prevent the spread of zebra mussels west of the 100th meridian (Britton and McMahon 2005). The larger the survey, the more accurate the predictions will be, especially because most boaters travel very short distances. For the 53,559 surveys used by Buchan and Padilla (1999), only 8.5% of all boaters traveled more than 50 km among lakes.

Surface-water connected lakes.—Because lakes connected by surface water are usually in close geographic proximity, they are likely to have similar geological features, water chemistry, and other factors that influence their suitability for zebra mussels (Koutnik and Padilla 1994). However, ground water and local geology can affect water bodies such that even though two lakes are connected they may not be equally suitable for zebra mussels. In that case, a downstream lake could act as a sink, preventing the spread of zebra mussels (Horvath *et al.* 1996). However, because water chemistry is an intrinsic feature of a lake, manipulating it is not a potential means of control.

River systems.—Because of the water continuity of a river system, it is likely that all sections of it will have similar chemical features, although substrate, productivity, and turbidity may change along it. Some sections of a river system may have features that make them more suitable for zebra mussels than others (Allen and Ramcharan 2001, Stoeckel *et al.* 2004b). Again, because these are intrinsic features of individual river systems, they are unlikely to offer avenues of control.

Step 3. The individuals must survive transport

If conditions during transport are unfavorable or transport time is too long, then survival during transport will be diminished. If individuals (such as planktotrophic larvae) require food during transport, then conditions must be appropriate for their potential food and there must be sufficient food for the needs of the individual. In addition, there must be adequate oxygen and the environment must be within the physical and chemical tolerances of the transported life stage. Although we have good data on the physical and chemical tolerances and physiological ecology of adult zebra mussels (reviewed by McMahon 1996, Karatayev *et al.* 1998), we have little information on tolerances of larvae.

Lakes with major shipping ports.—The conditions of ballast water tanks must be suitable for larval zebra mussel survival; there should be adequate oxygenation, appropriate thermal conditions, and no toxic chemicals. Transport time could also be an important factor. Wonham *et al.* (2001) found that the majority of species picked up in ballast do not survive transport. Zebra mussel larvae require adequate planktonic food for development and growth. Depending on temperature, they are in the plankton for 5 to more than 30 days (Sprung 1987, Stoeckel *et al.* 2004a). If temperatures are warm and there is adequate food, larvae could settle on the surface of the ballast tank inside a ship before they can be deposited in a new body of water.

Zebra mussels and other potential invaders could be controlled by altering conditions within ballast tanks. For example, removing oxygen from ballast water by bubbling nitrogen through the ballast tanks could effectively eliminate zebra mussels in the tanks, as they are extremely vulnerable to low oxygen levels (Tamburri *et al.* 2002). Many other chemical treatments have been suggested for cleaning ballast of invaders, each with its own limitations as described above.

Isolated inland lakes.—Transport over land is probably the harshest type of transport for either larval or adult zebra mussels. Larvae are unable to withstand desiccation, and therefore would be least likely to survive any transport when not completely immersed in water. Live wells in boats are small and subject to high temperatures and drying during transport, especially during summer. Adult zebra mussels are better able to survive desiccation during overland transport (Ricciardi *et al.* 1995), but cannot survive if they are out of water for too long (Johnson *et al.* 2001). In the former Soviet Union, where there are commercial fisheries in lakes with zebra mussels and fishing gear is transported among lakes, zebra mussels can survive extended periods of emersion attached to fishing nets or other fishing gear (Karatayev *et al.* 2003).

Surface-water connected lakes.—Transport among connected lakes will usually be the most favorable for survival of dispersing individuals. As long as water is flowing, the chemical, thermal, and biological attributes of connected waterways are often highly correlated with the source waters. Therefore, individuals capable of living in the source body of water should be capable of surviving in the water corridor connecting it with another body.

Control could only be effective if the water way did not flow continuously and was restricted or eliminated during times when larvae, the most likely dispersing phase, were in the water.

River systems.—River systems are also an excellent means of downstream, but not upstream, transport of larvae (Schneider *et al.* 2003, Carr *et al.* 2004, Stoeckel *et al.* 2004b), although shipping can be an effective means of upstream transport (Griffiths *et al.* 1991). As with other flowing water systems, adults will rarely be transported a great distance. Control of zebra mussel larval transport in rivers is challenging. Some recent work suggests that hydrodynamic turbulence, which affects larvae, could be an effective means of control (Rehmann *et al.* 2003), but this has yet to be tested in the field.

Step 4. The individuals (or their offspring if they reproduce during transport) must be deposited in the new location, and must survive until they can reproduce

If an organism is a specialist requiring a particular microhabitat or substrate, then deposition in an appropriate microhabitat or near an appropriate substrate is essential for settlement and growth. For zebra mussels, the main habitat requirements are for sites suitable for byssal thread attachment, and for well-oxygenated sites with suitable water chemistry. Similarly, local environmental conditions must be appropriate year-round for transported individuals to survive, reproduce, and become established. Zebra mussels can be limited by waters that are too cold or too warm (reviewed by Karatayev *et al.* 1997). Biotic interactions, especially the ability to escape from predators or parasites in their new habitats, could also be important (Johnson and Carlton 1996, Johnson and Padilla 1996, Karatayev *et al.* 1997, 1998).

Lakes with major shipping ports.—Adult zebra mussels attached by byssus to a boat hull or other parts of a boat are unlikely to be deposited into a new lake. If the boat is in the water and the animals are reproductive, however, they may spawn, releasing thousands of larvae into the water. Ballast water deposited in a port as a ship loads cargo will effectively deposit all live larvae (Grigorovitch *et al.* 2003). The larvae must then be capable of finding appropriate substrates for settlement.

Control could be effective if larvae were killed within the ballast tank (Tamburri *et al.* 2002) or upon leaving. Again, ballast exchange at sea or ballast sterilization could remove or kill freshwater larvae, preventing deposition at a freshwater port.

Isolated inland lakes.—As stated earlier, adult zebra mussels attached to a boat hull are unlikely to be deposited into a new lake, but may spawn and release larvae into the water. Waterweeds snagged on a boat trailer can float away from boats and trailers when deposited into the new site, delivering hundreds to thousands of adult zebra mussels (Johnson *et al.* 2001). For trailered boats, requiring that boats and trailers are washed and water weeds removed before allowing them to be put in the water would prevent this sort of introduction.

Surface-water connected lakes.—As long as water flows into a lake, larvae will flow with the water. If the lake has chemical or physical characteristics preventing zebra mussels from thriving or reproducing, then a new population will not form, even with continual input of larvae (Ramcharan *et al.* 1992a, Spada and Ringler 2002). There are no means of controlling zebra mussels in this situation. Clearing inflowing water of zebra mussels, without affecting other species, would be nearly impossible.

River systems.—River systems are very similar to surface-connected lakes. The chemical and physical conditions within the system must be adequate and there must be suitable substrate for larval zebra mussels to settle. Some river systems have very limited hard substrate or have large sediment loads, resulting in lower survivorship or success of zebra mussels (Karatayev *et al.* 1998). Control at this stage in the invasion process may also be difficult. For systems of locks and dams, such as along the Mississippi, attempts could be made to kill larvae as water is held in locks; however, selectively killing just zebra mussel larvae would be very difficult. In flowing river systems, zebra mussels have a metapopulation structure, with downstream populations dependent on the transport of larvae from upstream (Schneider *et al.* 2003, Stoeckel *et al.* 2004b). If one region of a river system was feeding larvae to the rest of the system, then intense control at source centers could result in eventual loss of zebra mussels from the system.

Step 5. The individuals must reproduce successfully and there must be continued reproduction, recruitment, and survival to establish a sustained population in the new location

For successful reproduction, free spawning aquatic animals must be in close proximity to compatible potential mates, and spawning must be synchronized so that sperm can effectively fertilize eggs (Levitan and Petersen 1995). In addition, larvae must develop, grow, and for benthic organisms, find adequate places for settlement and metamorphosis, again in close enough proximity to others such that when they grow up they also will be able to reproduce successfully (Grosberg and Levitan 1992).

Initial populations of most introduced species, including zebra mussels, would be expected to be small, and may remain small for extended periods, thus suffering high risks of extinction and Alee effects (Wang *et al.* 2002), as has been seen in the invasion of the saltmarsh grass *Spartina alterniflora* in Pacific coast marshes (Davis *et al.* 2004). When populations are very small, successful reproduction can be limited because of the difficulty of successfully finding mates, or, for free spawning species, successful fertilization of gametes. This limitation can affect the ability of populations to grow or to be sustainable, and can result in the extinction of small populations. Strong Allee effects can set threshold population sizes below which a population cannot grow. Invasive species frequently follow the pattern of having low population sizes for long periods before suddenly increasing, a pattern consistent with Allee effects. This pattern is often observed for populations of zebra mussel (reviewed by Karatayev *et al.* 1997). After initial low population levels, followed by increase, zebra mussel populations may remain high, decline to some lower, somewhat stable level, or oscillate through time (Ramcharan *et al.* 1992b).

Lakes with major shipping ports.—For large lakes with ports, Allee effects are likely to be important, but as long as there are plentiful substrates for attachment and appropriate physical conditions for zebra mussels, with time they will most likely be able to survive and reproduce. As with all organisms, zebra mussels are most vulnerable to control while populations are small and vulnerable to extinction. But, once populations are large, they will be virtually impossible to control.

Isolated inland lakes.—Isolated inland lakes will also have the limitations of population density and successful reproduction. However, in smaller lakes we might expect fewer or weaker Allee effects because gametes and larvae will be diluted to a lesser extent than in large lakes. This will allow the development of dense local populations more quickly, enhancing local reproductive success. As with large lakes that have shipping, populations are easier to control when densities are low, but nearly impossible to control when populations are large.

Surface water-connected lakes.—Surface water connection can help speed development of local populations because Allee effects will be lessened. The development of a local population will not be solely dependent on local larval production, but will be subsidized by upstream populations. Depending on local water retention times, however, loss of larvae downstream could be substantial. In that case, Allee effects could be stronger, increasing the time to development of a sustained population and increasing the likelihood of extinction. As long as the uppermost lake in a system has a permanent population of zebra mussels, the constant influx of larvae will continually reseed downstream populations even with local extinction.

River systems.—In unidirectional flowing water, problems of sperm dilution and effective fertilization for free-spawning animals like zebra mussels could be extreme (Levitan and Petersen 1995). River systems are structured as metapopulations. Downstream areas are always dependent on upstream populations for larval delivery, and local larval production will not be retained, but will be swept downstream (Schneider *et al.* 2003). Therefore, maintenance of a population of zebra mussels in a river system is dependent upon a permanent upstream population to act as a source of larvae. For example, in the Mississippi river system, Lake Pepin, an in-line "lake," seems to be an important source of larvae for areas downstream. Zebra mussel larvae are absent from plankton samples upstream of Lake Pepin and larval abundance peaks below it (Stoeckel *et al.* 2004b). Elimination of upstream populations that are the sources of larvae downstream would be an effective means of control and would result ultimately in loss of mussels from the whole system.

Estuarine rivers with bidirectional flow, however, present the possibility for local retention of gametes and larvae (Carr *et al.* 2004). In estuarine rivers, tidal forcing reverses water flow, trapping gametes and larvae within the system for extensive periods rather than sweeping them downstream. If net water flow out of the system is slow, then mixing could allow effective fertilization. And, depending on development time, larvae could be retained in the river rather than being swept downstream. This could allow local, self-replacing populations to develop. Bidirectional water flow will make control of zebra mussels very difficult, as populations should develop higher densities more quickly.

CONCLUSIONS

Zebra mussels offer an unusual opportunity to examine each of the steps in the invasion process. However, even for zebra mussels, quantitative data on spread and basic demography from different types of bodies of water are lacking. Future progress will depend on obtaining quantitative data on the transport and success of different life stages during each of the different stages of invasion. Thus far we can predict that developing controls on zebra mussels must be system-specific. Different types of bodies of water will have different vulnerabilities, and different stages in the life cycle and different stages in the invasion process will provide opportunities for control. Understanding both the life history of the invader and the properties of the habitat being invaded are critical for preventing or controlling invasion. For lakes with major shipping ports, such as the Laurentian Great Lakes, prevention of delivery of larvae through ballast seems the most effective and important point for control of introductions of zebra mussels of other freshwater species. For isolated inland lakes the adult stage is the most important; primarily adults attached to boats, anchors, and trailers moved among lakes. Requirements that boaters clean boats and trailers of attached adult mussels and water weeds when removing them from a lake with zebra mussels and before they are placed in a lake without zebra mussels could effectively control the spread of zebra mussels. For lakes with surface water connections the challenge is much greater when an upstream lake is invaded and larvae continually flow downstream. Therefore, focusing efforts on prevention of invasion in upstream lakes will be critical. Elimination of zebra mussels downstream will be impossible as long as there is a constant upstream source of larvae. The same holds true for river systems. With unidirectional flow, downstream populations will be dependent on larvae produced upstream for the maintenance of local populations. Elimination of the upstream permanent population will eventually result in eliminating zebra mussels from the system. Estuarine rivers with bidirectional flow pose a greater challenge because larvae may be retained in the system, allowing the development of self-recruiting populations.

Zebra mussels are important invaders in fresh waters around the world. As with most invaders, prevention of introduction is the best means for control. Once populations have become established, it is very difficult to eliminate them, but measures can be taken to slow the mussels' spread.

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Recently recognized risk of importing the giant African snail, *Achatina fulica* Bowdich, 1822, and its relatives into the United States and the efforts of the U.S. Department of Agriculture to mitigate the risk*

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Abstract: An infestation of the giant African snail, *Achatina fulica*, was established in North Miami Beach, Florida, in the 1960s as a result of the actions of a boy returning from Hawaii with three juvenile snails in his pocket. Seven years' efforts and $700,000 were required to eradicate the snails. More recently, *A. fulica* and two other achatinid species have become established on some of the Caribbean islands that trade with the United States, resulting in potential new pathways for introduction of these species. The Plant Epidemiology and Risk Analysis Laboratory (PERAL) of the United States Department of Agriculture (USDA) has prepared a pathway risk assessment to determine where resources should be allocated to prevent another infestation in the U.S. or its territories in conjunction with the recognition of these new risks. In the past, the number one pathway for importing achatinid snails into the U.S. had been considered to be deliberate smuggling of live snails in personal baggage from several countries in Africa and from Hawaii (based on data from internal USDA databases that track passengers and their possessions), but these newly recognized potential pathways from the Lesser Antillean islands of the Caribbean demonstrated a need for this new pathway and risk assessment, which is currently undergoing review, with appropriate mitigations for those pathways.

Key words: Caribbean, pathways, Antillean islands, infestation

The giant African snail, *Achatina fulica* Bowdich, 1822, has been considered "the most important land snail pest in the world" (Mead 1961, restated by Mead 1979, Srivastava 1986, 1992, Karnatak *et al.* 1998) and is one of the largest, at up to 15 cm in length (Mead and Palcy 1992). Published records indicate that 239 plant species in 183 genera, including many of economic importance, are known to be damaged by this snail (Lange 1950, Mead, 1961, Balasubramanian and Kalayanasundaram 1974, Singh and Roy 1977, Raut and Ghose 1984, Thakur 1998), although the number may be as high as 500 (Watson 1985). However, the extent of damage to most of these plant species is not known (Mead 1961). Those plants that have been reported to sustain the greatest damage include breadfruit, cocoa, cassava, Indian marigold, papaya, peanut, rubber tree, and most members of the Leguminosae and Cucurbitaceae (Raut and Ghose 1984, Watson 1985).

Documented costs of infestations by *Achatina fulica* include loss of agricultural productivity, cost of management or control, lost opportunity to cultivate crops that can no longer grow in infested areas, elimination of native gastropods through competition, vectoring of metastrongylid and bacterial parasites that cause human disease, and of plant disease organisms that cause crop damage (Raut and Barker 2002).

Individuals of *Achatina fulica* can carry the rat lungworm, *Angiostrongylus cantonensis* (Chen, 1935), which in humans produces eosinophilic meningoencephalitis and can cause blindness and death (Wallace and Rosen 1969, Watson 1985). *Achatina fulica* can also carry the bacterium *Aeromonas hydrophila* (Chester, 1901) Stanier, 1943 [=*Aeromonas liquefaciens* (Asai, 1935)]. *Aeromonas hydrophila* has produced in humans a variety of bacterioses including osteomyelitis, septic arthritis, tonsillitis, and meningitis (USDA 1982). Watson (1985) associated these bacterioses with eating undercooked snail meat and Prociv *et al.* (2000) stated that "the major role of *Achatina* might be . . . as a source of human infection." Mucus on unwashed hands, unwashed lettuce, and snail-contaminated drinking water can also become sources of the bacteria or nematodes (Alicata 1965, Kliks and Palumbo 1992, Thomé 1993, Hwang *et al.* 1994, Prociv *et al.* 2000).

Mead (1961) suggested that the "nuisance factor" of *Achatina fulica* not be overlooked: "The snails multiply in such unbelievable numbers that they crawl all over and into everything, they crush or slip out from under foot almost wherever one steps, they cover things with their excreta and sticky slime trails, and they die in great quantities for various reasons and create rank odors. In this manner they . . . assume an importance which may far outweigh any damage they may do to the vegetation." This point has been reiterated by Poucher (1975) and Civeyrel and Simberloff (1996).

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INTRODUCTIONS

Achatina fulica is native to Kenya, but since the eighteenth century has been internationally distributed, primarily by human activity (Lambert and Tillier 1993). Harry (1966) stated that "snails of very large size are nearly always introduced deliberately, usually for food." Species transported to distant areas they would never have reached on their own have been called "traveling species" (Smith 1989, McKinney and Lockwood 1999, Robinson 1999). *Achatina fulica* has been transported both deliberately by people and inadvertently in or on cargo and containers (Cowie and Robinson 2003, and data from an internal U.S. Department of Agriculture [USDA], Animal and Plant Health Inspection Service [APHIS] database, the Port Information Network, which captures interception events).

Prior to the eighteenth century, *Achatina fulica* was largely confined to eastern Africa (Mead 1961, 1979). Its use as a local source of protein resulted in widespread movement about the African continent by humans and subsequent introduction outside of Africa (Esobe 1986, Imevbore 1990, Wullschleger 2001). It was introduced (or first reported) in Madagascar in 1761, India in 1847, Malaysia in 1922, Indonesia in 1928, Philippines in 1932, Japan in 1933, and Hawaii in 1936 (Srivastava 1986, Mead and Palcy 1992, Raut and Barker 2002). More recently, introduction and establishment has occurred in the Lesser Antillean islands of the Caribbean: Guadeloupe in 1984, Martinique in 1988, and subsequently Barbados and Saint Lucia (Raut and Barker 2002, E. Ambrose pers. comm.). It became established in Brazil in 1997, with the states of São Paulo, Rio de Janeiro, Minas Gerais, Paraná, and Santa Catarina infested as of 2003 (Raut and Barker 2002, Coltro 2003). The U.S. mainland carries out extensive trade with these countries, so *A. fulica* and its relatives from all currently infested areas, in addition to Africa, were chosen for risk evaluation (Klassen in press, and an internal APHIS database—Agricultural Quarantine Inspection Monitoring).

ESTABLISHMENT AND ERADICATION IN THE UNITED STATES

An infestation of *Achatina fulica* was established in North Miami Beach, Florida, in the 1960s as a result of the actions of a boy returning from Hawaii with three juvenile snails in his pocket. In 1973, the State of Florida's Department of Agriculture and Consumer Services personnel declared *Achatina fulica* eradicated after more than 7 years of effort, expenditures of $700,000 and 67,000 person-hours, destruction of 18,000 snails, and distribution of 325,000 brochures mailed to the public (Poucher 1975). Intensive hand collecting of snails and over one million property surveys were carried out over a span of 5 years, and 25,000 infested properties in 9 separate infestation areas were treated with 128 tons [116 tonne] of molluscicidal baits. The property surveys involved infested and uninfested properties selected at random, with one-third of the properties being surveyed every two weeks, and surveys of all properties completed every six weeks (Poucher 1975). Properties were treated as many as 70 times (Poucher 1975). A subsequent estimate of the cost of doing nothing was $11 million per year (USDA 1982), which in 2003 dollars becomes $53 million, compared with $3 million (2003 dollars) for eradication (L. Garrett, pers. comm.).

ACHATINIDS INTERCEPTED ENTERING THE UNITED STATES

To counter the threat of pest infestations on the mainland U.S. or its Caribbean territories, the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), and Plant Protection and Quarantine (PPQ) inspectors at all ports of entry intercept (confiscate) organisms considered to be undesirable for the environment or agriculture in the U.S.

Table 1. Geographic origins of achatinids intercepted by PPQ, with numbers of interceptions, for each of four species from 1993 to 2003 (origins with one interception omitted). Total numbers of interception events are listed; actual numbers of snails intercepted may be higher, since some events included interception of multiple snails (extracted from internal USDA database).

Origin	*Achatina achatina*	*Achatina fulica*	*Archachatina marginata*	*Limicolaria aurora*
Africa	13		14	
Ghana	76	7	12	
Guam		1		
Hawaii		73		
Marshall Is.		1		
Nigeria	73		75	2
Senegal	11			
Other	15	2	1	

Achatina fulica has not been the only achatinid to be intercepted. Others include *Achatina achatina* (Linnaeus, 1758), *Archachatina marginata* (Swainson, 1821), and *Limicolaria aurora* (Jay, 1839), mostly from African countries. Intercepted individuals of *A. fulica* tend to have originated in Hawaii or other Pacific islands including Guam and the Marshall Islands (Table 1). The risk of people arriving with achatinids in their baggage is well enough known that flights originating from areas with an interception history are targeted for baggage inspection either via manual search or x-ray examination on the baggage floor (Data from an internal USDA-APHIS database, the Port Information Network, which captures interception events). Achatinids are recognizable as eggs, young, and adults, and the calcareous shells show up on x-ray.

Most flights originating in African countries with populations of *Achatina fulica* or other achatinids land at John F. Kennedy International Airport (JFK) in Jamaica, New York. All intercepted individuals of *Achatina achatina, Achatina marginata,* and *Limicolaria aurora* recorded between 1993 and 2003 arrived from African countries (Table 1). Therefore, these species are most likely to be intercepted at JFK (Table 2). *Achatina fulica* is established in Hawaii but is not established on the mainland U.S. or the U.S. territories of Puerto Rico and the U.S. Virgin Islands, so flights from Hawaii to mainland North America are subject to preclearance inspections (Table 2).

Most of the interceptions summarized above were of snails that were ultimately to be used for food (Moore *et al.* 1998). Table 3 gives a perspective on the magnitude of the task of inspecting air passengers arriving at JFK, and stopping *Achatina fulica* from entering. Other ports of entry present similar difficulties to inspectors trying to find and intercept prohibited organisms. Inspectors are not able to intercept all invasive organisms that enter the U.S. (Klassen in press).

Table 2. Major ports of entry into the mainland US and numbers of interception events for each of four species of achatinids intercepted by PPQ from 1993 to 2003 (internal USDA database: Port Information Network).

Port of entry	*Achatina achatina*	*Achatina fulica*	*Archachatina marginata*	*Limicolaria aurora*
Atlanta, Georgia	7		7	
Chicago, Illinois	9		9	
Dulles airport, Virginia	18		7	
Honolulu, Hawaii*		34		
Houston, Texas				1
JFK airport, New York	116		50	
Kailua Kona, Hawaii*		4		
Lihue, Hawaii*		34		
New Orleans, Louisiana				1

* Hawaiian interceptions resulted from preclearance inspection activities.

POTENTIAL ESTABLISHMENT OF *ACHATINA FULICA* IN THE UNITED STATES

Most achatinids are tropical/subtropical in their environmental requirements, but *Achatina fulica* is tolerant of somewhat more temperate conditions (Mead 1961, Zhou *et al.* 1998, Raut and Barker 2002). Based on data for individuals of *A. fulica* established in China (Zhou *et al.* 1998) and data of Raut and Barker (2002), extrapolations were made to predict the potential distribution of *A. fulica* in the mainland U.S. if an infestation occurred and no mitigation or eradication were carried out (Fig. 1). A map was generated from data given by the above authors: minimum survivable temperature 2°C (by hibernation), active temperature range 9° to 29°C, maximum survivable temperature 30°C (by aestivation) (Raut and Barker 2002); and minimum survivable temperature 0.2°C, temperature necessary for development 12°C, and maximum survivable temperature 41.2°C (Zhou *et al.* 1998). These data were extrapolated onto a grid via a global information system, matched to grids for the U.S. based on weather station data of minimum January and maximum July air temperatures, and queried for areas of commonality. Interpolations were made via an inverse distance weighting system (Fisher *et al.* 1987). Mead (1973) stated: ". . . it is predicted that *A. fulica* will eventually spread north to the Carolinas and west, through the Gulf states, spottedly through the Southwestern 'desert' states, and into southern California." The results of the climatic matching agree well with Mead's predictions. The destinations of travelers intercepted by PPQ with living achatinids between 1993 and 2003 (Table 4) suggest that the snails could have been distributed to numerous states, of which California, Florida, Georgia, and Texas are potentially suitable for *A. fulica* (Fig. 1).

RISK ASSESSMENT

To aid the USDA-APHIS-PPQ inspection effort, the USDA's Plant Epidemiology and Risk Analysis Laboratory (PERAL) performs pest risk assessments when requested both for particular organisms and their pathways of introduction. Risk assessments are used to help distribute resources in the most efficient way.

Achatina fulica and its relatives have been intercepted by PPQ, according to Port Information Network records (internal USDA-APHIS

Table 3. Numbers of air travelers entering the U.S. at John F. Kennedy International Airport (JFK) and numbers of prohibited pests, including achatinids, intercepted by PPQ (USDOT 2004; internal USDA database: Port Information Network).

Year	Total passengers[1]	African passengers[2]	Total pests[3]	African pests[4]	Achatinids[5]
2000	17,962,332	760,258	3,264	257	8
2001	15,573,810	729,936	3,977	173	10
2002	13,957,590	622,661	3,642	187	10

[1] All air passengers arriving at JFK.

[2] All air passengers arriving at JFK from African countries that have infestations of *Achatina fulica*.

[3] All PPQ pest interception events.

[4] All PPQ pest interception events made at JFK from African countries that have infestations of *Achatina fulica*.

[5] All PPQ achatinid snail interception events made at JFK.

database), since at least 1985. However, records prior to 1993 have not been used in the risk assessment because it was not until 1993 that PPQ hired an official malacologist identifier who checks the molluscan data entered into the Port Information Network for accuracy. Achatinids have been intercepted 362 times during this period, with the three species now present in the Lesser Antilles accounting for 181 (50%) of these interceptions (Table 5).

Achatinids account for just 3% (362 of 12,119) of all snail interception events recorded by PPQ. Of all snails associated with baggage, either as a plant pest, hitchhiker, or smuggling, achatinids made up 31% (354 of 1,149) of interception events. Achatinid species constituted 67% (317 of 476) of interception events of snails being deliberately smuggled (internal USDA-APHIS database).

Deliberate smuggling of live edible molluscs in baggage constituted the primary known pathway for achatinid entry into the U.S. prior to infestation of the Caribbean islands. Three achatinid species reaching the U.S. in baggage constituted 90% (317 of 352) of the interceptions for achatinids; *Limicolaria aurora* was intercepted twice during this period and eight interceptions were not identified to species (Table 5). Containers (2.5% or 9 of 352) and cut flowers (6% or 22 of 352) constitute less important known pathways of entry for these species into the U.S. (Table 5).

The risk assessment evaluates criteria such as origins of the species in question, the ports of entry in the U.S. where the species are most often encountered, and the destination within the U.S. of the species. These data were used to make risk evaluations that include mitigation recommendations to increase the chance of intercepting the species and preventing their introduction and establishment in the U.S.

A NEW RISK

No new risk analyses or mitigations would have been needed if the situation were static, but three of the achatinid species mentioned above are now present in the Caribbean Lesser Antilles. *Achatina fulica* appeared on Guadeloupe in 1984 and Martinique in 1988, generating concern because Martinique had been a major producer of cut flowers for export to the mainland U.S. (Raut and Barker 2002). *Achatina fulica* is now also in the islands of Barbados and Saint Lucia, and *Archachatina marginata* and *Limicolaria aurora* are in Martinique (Mead and Palcy 1992, E. Ambrose personal communication). Achatinids are also hitch-hikers on cargo containers, on fruits, vegetables, and other commodities (Table 5). Because the Caribbean countries are the tenth largest U.S. trading partner and entry of invasive species is correlated with trade and tourism (Klassen in press), there is probably an increased risk that U.S. trade with these islands could lead to another establishment of these pests. Fishing craft and pleasure boats travel into the Caribbean, presenting another potential pathway. In addition, *A. fulica* became established in Brazil in 1997 and is now widespread there, creating an additional threat.

THE NEW RISK ASSESSMENT

In response to these new threats, the possibility of new pathways for entry of achatinids into the U.S. mainland, and the need for changing priorities in equipment and personnel whose job it is to intercept prohibited organisms, the new risks of this threat are under review. PERAL has prepared a “Pathway Risk Assessment for Achatinidae with Emphasis on the Giant African Snail *Achatina fulica* Bowdich, *Limicolaria aurora* (Jay), and *Archachatina marginata* (Swainson) from the Caribbean and Brazil,” which is currently undergoing review. Because *A. fulica* is already established in Hawaii, the assessment for this species includes all U.S. territories except Hawaii.

Data available to USDA (internal databases used to track port interceptions and movement of goods and people into the U.S.) are insufficient to support quantitative risk analysis for the threats presented by infestations of *Achatina fulica* in the Caribbean. A non-quantitative process has been developed by USDA-APHIS (Orr *et al.* 1993) using the procedures of Kaplan (1992) that make use of “expert information” and “expert opinion” to provide risk analyses for such situations. These methods were followed for an analysis of pink mealybug, *Maconellicoccus hirsutus* (Green, 1908), from the Caribbean (Chang and Miller 1996). *Maconellicoccus hirsutus* has since become established in Florida (Feiber 2004). The template described by Chang and Miller (1996) has now

Table 4. Destinations of travelers carrying achatinids intercepted by PPQ, based on interviews conducted where travel baggage was retrieved; for interceptions from 1993 to 2003 (internal USDA database: Port Information Network).

	NY	CA	NJ	TX	GA	IL	MD	MA	VA	FL	Other	Total
Number	144	51	24	23	19	16	11	10	10	9	44	361
Per cent	40	14	7	6	5	4	3	3	3	2	12	99[1]

NY, New York, CA, California; NJ, New Jersey; TX, Texas; GA, Georgia; IL, Illinois; MD, Maryland; VA, Virginia; FL, Florida.

[1] Rounding errors contributed to a total of less than 100%.

been used to develop analyses of pathway risks for *A. fulica* from the Caribbean and Brazil.

Potential pathways from the Caribbean and Brazil that are being considered in the risk analysis include (1) express packages, (2) cargo ships that dock in Miami or other Florida ports, (3) empty containers carried by cargo ships, (4) cruise ships, (5) pleasure and fishing craft, and (6) the pet trade.

A potential additional contributing factor is trade carried out by Puerto Rico and the U.S. Virgin Islands. Based on 2002 data, Puerto Rico received 10,181 shipments of consumables such as fruits, vegetables, and cut flowers, and of this total, 2,129 (21%) were shipped from elsewhere in the Caribbean. The U.S. Virgin Islands received all of its 572 shipments of these consumables from elsewhere in the Caribbean. Without mitigation in the islands that now have achatinid infestations, spread of these snails to these U.S. territories is expected (Klassen in press).

Express packages

Express packages constitute a pathway for goods from Brazil. In 2003, 12% of the packages x-rayed on the package floor contained contraband–illegal drugs and antibiotics (J. Aliaga, W. Tang, and F. Zimmerman pers. comm.). However, since five states of Brazil were infested with *Achatina fulica* (see Coltro 2003) and since achatinids are considered to be food by certain cultures (Harry 1966, Robinson 1999), this pathway may develop in importance. At present, it is rated low, with the recommendation that it be monitored for changes that could lead to importation of *A. fulica*.

Cargo ships and containers

Cargo ships and containers are considered together. Several small shipping companies have container yards on the Miami River and dock small container ships there. At least one of these shipping companies has ships that regularly travel to the Lesser Antilles. These ships do not travel just to Barbados, Guadeloupe, Martinique, and Saint Lucia, but also stop at other ports on the way north, including the U.S. territories of Puerto Rico and the U.S. Virgin Islands. One such company estimated that it returns 1,500 containers per month back to Miami from the Lesser Antilles and another 1,500 to other ports in Florida (J. Aliaga, W. Tang, and F. Zimmerman pers. comm.). Most containers were empty returning to the U.S., but about 2% also carried cargo that could serve as a pathway. Table 6 illustrates the size of the Caribbean and Brazilian commodity and goods trade pathway (USDA internal database), which doubled from 1994 to 2000, while during the same period interception of pests of quarantine significance increased nearly four-fold, overwhelming the capacity of inspectors to check incoming products thoroughly (Klassen in press).

D. G. Robinson and W. Tang (pers. comm.) determined that the container pathway is significant for snails other than achatinids. Although adult achatinids may be too large to escape detection, containers traveling from infested Caribbean islands must be considered as a pathway, at least for hatchling snails. Overall, container yards are an inhospitable environment for hitchhiking snails, but those on the Miami River have weedy margins and lushly inhabited riverbanks where snails could become established. *Achatina fulica* has been reported damaging 239 plant species (see above), many of which occur in Florida. This pathway must be considered as potentially increasing, since Florida serves as a point of origin or destination for most shipments between the U.S. and the Caribbean (Klassen in press), and

Table 5. Numbers of interceptions of achatinid species intercepted by PPQ from January 1993 to June 2003, sorted by pathway of entry. Total number of interception events are listed; actual numbers of snails intercepted may be higher, since some events included interception of multiple snails.

Species	Baggage	Containers	Foliage	At large	Total
Achatina achatina	164	5	3	1	173
Achatina fulica	56	3	19	1	79
Archachatina marginata	97	1	0	2	100
Limicolaria aurora	0	1	1	0	2
Undetermined to species	5	1	1	1	8
Total	322	11	24	5	362

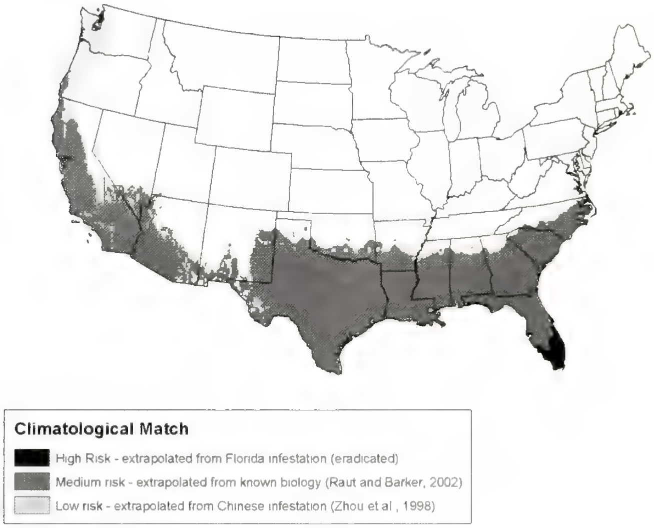
Climatological Match
High Risk - extrapolated from Florida infestation (eradicated)
Medium risk - extrapolated from known biology (Raut and Barker, 2002)
Low risk - extrapolated from Chinese infestation (Zhou et al , 1998)

Figure 1. Predicted Distribution of *Achatina fulica* if permitted to become established without mitigation in the mainland U.S., based on parameters extrapolated from Zhou *et al.* (1998) (light gray area = low risk), Raut and Barker (2002) (dark gray area = medium risk), or the eradicated Florida infestation (black area = high risk).

once an invasive species gains a foothold anywhere in the greater Caribbean basin, it tends to spread throughout the region (Darling *et al.* 2001).

The method of mitigation preferred by the USDA for removing *Achatina fulica* from this pathway is prevention of infestation at the ports of lading and the container yards on those Caribbean islands already having infestations of *A. fulica,* since pest risk mitigation at the site of origin is considered to be the most effective method (Klassen in press).

Cruise ships

Cruise ships carry all their produce from U.S. ports and inspect passengers for live materials, so they are considered low risk. Trinkets picked up on the beach may represent a pathway, however.

Table 6. Number of shipments of air and maritime cargo arriving in Miami with Caribbean or Brazilian origins (internal USDA database: Port Reports for Commodity and Goods Movement).

Origin	Air cargo	Maritime cargo	Period
Barbados	524	658	December 1997-May 2003
Guadeloupe	26	0	November 2001-July 2002
Martinique	4	3	November 2001-July 2002
Saint Lucia	1572	157	November 1997-May 2003
Brazil	**	8162	May 2002-May 2003

** not reported in the database

Pleasure and fishing craft

Pleasure craft visiting the Lesser Antilles are considered to be of low risk because most of the craft make numerous stops returning to Miami from the Lesser Antilles (personal interviews with Miami boat slip workers) and fewer than three dozen pleasure craft arriving in Florida per year have traveled to the Lesser Antilles. Fishing boats are mostly small to medium sized craft, the range of which would not allow them to travel to the Lesser Antilles, so they were also estimated as low risk (J. Aliaga, W. Tang, and F. Zimmerman pers. comm.).

Pet trade

The pet trade is regulated by more than one government agency, but instances of smuggling have been documented (Britton 1993, Schmitz and Simberloff 2001). Wholesale importers and retail pet stores culture, transport, and sell non-native fresh and salt water plants, fish, and invertebrates. Individuals who do not follow strict protocols for animal management may accidentally allow escape or intentionally release organisms from confinement. The intentional and unintentional release or escape of organisms into the wild by both industry and the hobbyist aquarium owner has led to introductions and establishments (Moore *et al.* 1998). The same scenario may obtain for achatinids. The pet trade for exotic aquarium and terrarium organisms has been estimated to generate $25-30 billion per year in the U.S. and online shopping is increasing in popularity, so the potential of this pathway would appear to be increasing (Maeda *et al.* 2000). Even the most efficacious quarantine regulations can fail when determined individuals make concerted efforts to circumvent them (Mead 1979).

People in the U.S. seem to have an insatiable appetite for new and exotic pets (J. Aliaga, W. Tang, and F. Zimmerman pers. comm.; Anonymous 2003), which feeds this pathway. At least four regulatory incidents have resulted from people importing live achatinids for the express purpose of rearing and distributing them (Jones 1969, McLawhorn and Habeck 1992, Thomas 1995). This pathway may be more problematic than others primarily because purchasers of pet snails have in mind deliberately keeping the snails alive and establishing colonies (Cowie and Robinson 2003), and because distributors outside the U.S. may be unfamiliar with the import laws governing live organisms. A recent survey of 101 schools, 133 pet stores, 12 exotic animal shows, 12 animal swap meets (shows where buyers and sellers trade live animals), 19 miscellaneous markets, and a Puerto Rico pre-clearance airport inspection by officers of USDA's Safe-

Table 7. Numbers of specimens of *Achatina fulica* seized by Safeguarding Intervention and Trade Compliance officers during a survey of schools, pet stores, and animal shows in seven states and a Puerto Rican airport (internal USDA database: Safeguarding Intervention and Trade Compliance).

	Illinois	Indiana	Michigan	New Jersey	Ohio	Pennsylvania	Puerto Rico	Wisconsin	Total
Number of cities	7	4	6	1	4	1	1	37	61
Seizures	98	300	717	1	3,139[1]	170	1	1,364	5,892

[1] One seizure resulted in 2,500 specimens, which the owner stated were the offspring of one adult snail.

guarding Intervention and Trade Compliance branch illustrate the potential for this pathway (Table 7).

CONCLUSIONS

Historically, achatinid snails have been imported for food by people arriving on flights originating in Africa, Hawaii, and other Pacific islands; and the predominant ports of entry were the airports at which such flights entered the U.S. (Tables 1, 2, 8). The risk situation may have changed with the infestation of four Caribbean islands with one or more species of achatinid snails (E. Ambrose personal communication), as well as establishment of *Achatina fulica* in Brazil. Trade carried out by Florida, Puerto Rico, and the U.S. Virgin Islands, has presented several potential pathways not previously recognized. The main pathways include express packages from Brazil, cargo ships and containers returning from the Lesser Antilles, and the pet trade.

Mitigation measures include taking a closer look at these pathways, determining the level of risk they present, and management of populations of *Achatina fulica* in the Caribbean islands that ship to the U.S. or its territories. The threat to the mainland U.S. would increase dramatically if the spread of *A. fulica* and its relatives is allowed to go on unabated through the Caribbean Basin and result in greater risk that another infestation of *A. fulica* would have to be eradicated in Florida or in the U.S. territories of Puerto Rico and the U.S. Virgin Islands. Table 8 summarizes the historical situation. The currently changing pathway risks are being watched and evaluated by USDA to determine what actions are needed to mitigate the new risks and close the new pathways. People and resources have been and are being redirected based on the perceived new risks, which cannot yet be quantified but which are nevertheless based on expert information (Kaplan 1992).

Table 8. Summary of the major known pathways, origins, ports of entry, and destinations, given as percentages in the United States, for three species[1] of achatinid snails intercepted by PPQ (internal USDA database: Port Information Network).

	Achatina achatina	*Achatina fulica*	*Archachatina marginata*
Pathway	95% baggage	71% baggage	97% baggage
Origin	99% African	94% Pacific Is.	99% African
Port of Entry	67% JFK[2]	91% Hawaii[3]	50% JFK[2]
Destination	57% New York	60% California	43% New York

[1] *Limicolaria aurora* was omitted from this table because it has been intercepted only twice.

[2] John F. Kennedy International Airport.

[3] Hawaiian pre-clearance stations including Honolulu, Kailua-Kona, and Lihue.

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Invasion and evolution: Why do herbivorous and carnivorous land snails invade but not originate on islands?*

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Abstract: Species such as the giant African land snail (*Achatina fulica*) with high competitive and reproductive performance do not evolve on islands but are successful invaders once humans have helped them become established there. I suggest that ocean barriers, the small size of islands and the related low standard of performance among endemic insular species, and the apparent absence of herbivorous and carnivorous habits among endemic island snails, which are generally fungivorous and detritivorous, are three factors that together provide an explanation for this observation. A test of this hypothesis will require a much more detailed knowledge of the natural history of land snails in source regions and on islands than we now have.

Key words: Gastropoda, herbivores, predators

Island ecosystems under human hegemony have been famously affected by an army of invading plant and animal species deliberately or unintentionally brought by people from far-away continents. If these invaders are so successful in the recipient biotas of these islands, why did they not arise there in the first place? Why did an herbivorous mammal or bird with the competitive and reproductive characteristics of the European rabbit not evolve in the island-like continent Australia, where the rabbit has been spectacularly successful? Why did the native flora of Guam not produce a nitrogen-fixing species like *Leucaena leucocephala* (known as the tangantangan in Guam), a prolific Central American small tree introduced by the U.S. military administration following the ravages of World War II? And why did *Achatina* Lamarck, 1799, the large-bodied African land-snail genus, evolve in continental Africa and not on oceanic islands, where it has enjoyed enormous success and where, as Solem (1973a) pointed out, native land-snail diversity in the absence of predators is often strikingly high?

Here I want to suggest a three-part answer to these questions. The first part of the answer, I believe, has to do with how terrestrial species cross hostile ocean barriers unaided by humans. For a variety of reasons, species with rapid metabolic rates and high competitive and reproductive performance are less apt to invade islands from continents than species with more modest capacities of performance. The second has to do with the conditions of life on islands as compared to mainlands. Islands support small economies in which the scope for the evolution of species with high performance levels is very limited. Finally, the third part is specific to the biology of the invaders in question. For reasons that are not entirely clear, *Achatina fulica* Bowdich, 1822, and other highly destructive land-snail invaders brought by humans to islands are either herbivores or active predators, in contrast to most island endemic land snails, which are low-energy consumers of fungi or detritus.

BARRIERS

A barrier separating two suitable environments prevents dispersal of individuals because conditions are hostile there. In order to cross the barrier, an individual must have one or more of the following attributes: (1) the capacity to feed during some life stage during the crossing; (2) the wherewithal to store and use energy reserves during the crossing; (3) the ability to shut down metabolism; and (4) the ability to avoid the objectionable conditions by hitching a ride on another organism or by traversing the barrier under the individual's own power.

The criteria stack the deck against mammals, which neither shut down for long periods (except for some hibernating bears, rodents, and bats) nor swim or fly across wide stretches of ocean (e.g., Ziegler 2002). Barriers pose fewer hurdles for lizards, water-resistant plant seeds, wind-dispersed fern spores, flying birds, and land snails. Although salt water is inimical to them, many land snails can suspend most metabolic activity for extended durations, and have the ability to attach to objects or animals while hermetically encased in a shell sealed by an operculum or epiphragm.

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ISLAND CONDITIONS

Under island conditions without human influence, there are severe limits placed on the per-capita and population-level competitive and reproductive performance of species by the island's small size. As noted by Burness *et al.* (2001), species with a high per-capita food requirement and a high metabolic rate cannot maintain populations of as many individuals as species with lower requirements. Large mammals have individual requirements so high that the maximum population size possible on a small island is low enough that the population is vulnerable to stochastic extinction. For such high-energy animals, viable population size is attainable only if individual demand decreases, either through reduced metabolic rate or reduced body size or both (Burness *et al.* 2001). Island conditions without human tampering thus reinforce the selectivity of the dispersal barrier in generally favoring species with low individual and population-level demands.

And yet, high-energy or large-bodied invaders arrive, and when they do so they do well on islands. Why then, don't organisms with those characteristics evolve there? There are at least three complementary explanations. First, in the long term, these species may not last, even if in the short term they do well. Stochastic extinction events may be common, but they do not occur every day. High-energy populations may therefore be eliminated decades or even centuries after their introduction to the island. Second, given that the standard of performance is in absolute terms lower in the small biotas and the small habitat areas of islands than in the large expanses and biotas of continents, a newly arising endemic species does not have to equal the best that a continent has to offer; in order to be the local competitive dominant, all the species has to do is to surpass the highest performance level of already existing island incumbents. There is no advantage in exceeding this threshold very far; indeed, there may be disadvantages. Competitive dominance at the level seen in continental species comes at a high price, which may be worth paying where the stakes of competition are high, but not in island situations where the stakes are lower (see many early references in Simberloff 2000). Third, when vigorous species arrive with people on islands, they become established in disturbed agricultural or horticultural settings that humans have made highly productive and perhaps therefore more like the environments in which these species evolved.

THE CASE OF LAND SNAILS

How do these general ideas apply to the particular case of invasive land snails? The first point to make is that, although land snails as a group are good candidates for crossing ocean barriers from continents to islands because of their low metabolic rates and their ability to withstand inclement conditions for long periods, island faunas reveal that instances of invasion have been rare. As noted by Cameron and Cook (1989) for Madeira, Cowie (1995, 1998) for the Hawaiian Islands, and Bieler and Slapcinsky (2000) for Bermuda, most of the observed high diversity of land snail biotas on oceanic islands is the result of *in situ* speciation, not of repeated invasion. Evidently the combined challenges of crossing a wide hostile barrier and of establishing a viable population in a recipient biota are substantial even for groups that are as well predisposed as are land snails.

The kinds of snails that humans have introduced to islands, moreover, differ importantly both from the snails that have invaded islands without human help and from those that evolved on the islands themselves. The snails that are widely perceived as invasive island pests belong to two categories that are either wholly missing in endemic island faunas or very poorly represented there. These are species that eat living green plants (e.g., *Achatina fulica*) and those that eat other land snails (*Euglandina rosea* Férussac, 1821, and species of *Gonaxis* Taylor, 1877). Detritivorous subulinids are often abundant invasive snails on islands, and may compete with the local endemics (Cowie 2001a, b), but they are not generally considered pests.

Most terrestrial gastropods throughout the world live in the leaf litter; where there is limestone, many live on rock; and in warmer regions there is a fair diversity of tree-dwelling species. We know appallingly little about the food habits of land snails, but the available evidence indicates that most of the snails in leaf litter eat detritus and fungi, whereas rock-dwellers and tree-climbing species tend to feed on lichens and algae (see e.g. Dimelow 1962, Solem 1973b, Severns 1981, Tuskes 1981, Baur *et al.* 1994, Heller and Dolev 1994, Hesbacher *et al.* 1995).

Although few if any terrestrial gastropods exclusively eat living plants, the herbivorous habit has evolved a number of times among mainly Old World groups (we know much less of the feeding habits of New World groups). In addition to dead plant matter, living plants are consumed by species of the giant African land snail genera *Achatina* and *Archachatina* Albers, 1850 (Okafor 1989, Monney 1992, Duah and Monney 1999), European larger helicids (Frömming 1953, Chatfield 1973, Williamson and Cameron 1976, Heller and Ittiel 1990, Speiser and Rowell-Rahier 1991, Linhart and Thompson 1995, Westerbergh and Nyberg 1995, Iglesias and Castillejo 1999, Chevalier *et al.* 2000), European and Asian bradybaenids (Dundee and Cancienne 1978), and various Old World slugs (Frömming 1953, Westerbergh and Nyberg 1995, Frank 1998). I am unaware of any endemic insular land snail whose diet contains living plants. Invaders such as *Achatina,* larger helicids, and limacid and arionid slugs therefore filled a trophic role not filled by any native insular gastropod.

Predatory snails have also arisen many times independently (Solem 1974, Hausdorf 1998). Land snails that eat other land snails are known from all continents except Antarctica, as well as from New Zealand and New Caledonia. Examples of predatory snails that have become pests after being introduced by people to islands include the spiraxid *Euglandina rosea* (originally from Florida) and African species of the streptaxid genus *Gonaxis. Plutonia atlantica* (Morelet, 1860) in the Azores appears to be exceptional among island endemics in being an island species that acquired its predatory habits on an oceanic island (Medeiros *et al.* 2000). The predatory land snails of New Zealand and New Caledonia likely date from a time when these continental fragments were part of the large continent of Gondwana (see Stilwell 2003).

It is quite unclear to me why live-plant herbivory and predatory habits should not have evolved among endemic land snails on islands. Perhaps these habits on continents are associated with high fecundity and rapid growth rates. Certainly some island species such as Hawaiian achatinellines (Hadfield *et al.* 1993) and Pacific island partulids (Cowie 1992) have very slow growth rates, characteristics that with some exceptions, such as some endemic Hawaiian succineids (Rundell and Cowie 2003), may be more common among island snails than among those of continents.

The apparent failure of insular land snails to evolve herbivorous or predaceous habits raises several other unanswered questions. First, what is the situation in endemic clades of other major groups such as vertebrates and insects? I am too ignorant of the entomological literature to even hazard a guess, but among vertebrates, enhanced tendencies toward herbivory seem to have evolved repeatedly in New Zealand (moas), the Hawaiian Islands (moanalos, now extinct), and the Mascarene Islands (various pigeons) (Olson and James 1991, Livezey 1993, Worthy and Holdaway 2002). Second, why is obligate consumption of living vascular plants so rare even among continental land snails? Which factors have prevented herbivorous land snails not just from taking only green plants, but from undergoing the kind of trophic specialization that characterizes the vast majority of herbivorous insects? A third question is whether land snails with life styles unlike those of native insular species could have become established on islands in the absence of human-caused habitat disruption. Even if such snails could have made the transoceanic journey on their own, could they have invaded an endemic island biota that had not already been ravaged by extinction or driven into relictual fragments, many at high elevations? Cowie (2001b) noted that established land-snail invaders have been able to penetrate native habitats in the Hawaiian Islands and elsewhere, and the predatory *Euglandina rosea* has brought about the extinction of perhaps hundreds of endemic island snail species throughout the Pacific.

Even if answers to these questions remain elusive at present, the questions suggest an agenda for future work on invasive land snails. Instead of emphasizing diversity as the dominant measure by which we evaluate the effects of invasion, we should shift attention to the ecological and evolutionary context of invasion. This means characterizing the species pool in source regions, studying the biota into which immigrants become established, understanding the selectivity of the ocean barrier, and considering how the species of interest interact not just with other members of the larger clade to which they belong—various clades of land snails in this case—but also with species in other major clades.

In the introduction, I asked why giant African land snails do not evolve on islands. As a hypothesis, the answer is that the ocean barriers, limited size of insular ecosystems, and limitations related to trophic roles conspire to prevent snails with the adaptive characteristics of invasive continental land snails to evolve in isolated insular settings. A test of this hypothesis will shed much-needed light on the biology of land snails of both islands and continents and point the way to additional promising questions.

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Analysis of trailered boat traffic and the potential westward spread of zebra mussels across the 100th meridian*

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Abstract: The 100th Meridian Initiative is a cooperative effort between federal, regional, tribal, and local agencies/organizations devoted to preventing the westward spread of zebra mussels and other aquatic nuisance species in North America. Between 1998 and 2002 participants of the 100th Meridian Initiative conducted boater interviews in several states bisected by and west of the 100th Meridian. Data obtained included origins and destinations for trailered boats. Boats visiting areas within 40 km of known zebra mussel infestations of zebra mussels were considered to be potentially contaminated because zebra mussels can attach to boats and survive several days out of water under favorable conditions. Thus, other destinations to which such boats are trailered are possibly at risk of invasion by zebra mussels. Based on boater surveys, bodies of water receiving the most boats from contaminated regions were found to be Lake Francis Case (South Dakota), Cheney Reservoir (Kansas), El Dorado Lake (Kansas), Table Rock Lake (Missouri), Winfield City Lake (Kansas), Lake Fork Reservoir (Texas), Grand Lake o' the Cherokees (Oklahoma), Lake Oahe Dam (South Dakota), Lewis & Clark Lake (South Dakota), and Keystone Lake (Oklahoma). Major highway segments where at least 500 surveyed boaters from contaminated areas crossed between 1998 and 2002 were Interstate Highway 90 (I-90), I-29, U.S. Highway 18, and State Highway 50 in southeast South Dakota; I-80 in southwest Nebraska, I-76 in northeast Colorado, and U.S. Highway 54 and State Highway 96 in south central Kansas. Thus, these bodies of water and highway segments are well suited for public outreach efforts to educate boaters about aquatic nuisance species, emphasizing the importance of inspecting and cleaning their boats to reduce the risk of unintentional dispersal of aquatic nuisance species to other bodies of water.

Key words: *Driessena polymorpha,* zebra mussel, GIS, 100th Meridian Initiative, aquatic nuisance species

The Western Regional Panel on Aquatic Nuisance Species, under the direction of the United States' Aquatic Nuisance Species Task Force, began an initiative in 1997 to prevent the westward spread of aquatic nuisance species across the 100th meridian (100° west longitude). This cooperative effort, named the "100th Meridian Initiative," was made possible under a provision in the U.S. National Invasive Species Act of 1996 (Public Law 101-636). Federal, tribal, regional, and local agencies of the United States and Canada, as well as private organizations, are involved in this effort, emphasizing the importance of thwarting further invasions by zebra mussels, *Dreissena polymorpha* (Pallas, 1771), a highly invasive freshwater molluscan species that is ecologically destructive and a major macrofouling species in industrial, potable water, and power station raw-water systems (United States Congress, Office of Technology Assessment 1993, O'Neill 1997).

Zebra mussels are of extraordinary concern because trailered boats and other hauled equipment can be a vector for their invasion of new bodies of water (Carlton 1993, Johnson and Carlton 1996, Johnson and Padilla 1996, Padilla *et al.* 1996, Schneider *et al.* 1998, Buchan and Padilla 1999, Kraft and Johnson 2000, Johnson *et al.* 2001). In infested areas, zebra mussels attach to practically any hard substratum experiencing prolonged submersion (Mackie 1991), including boats and boating equipment. Johnson *et al.* (2001) conducted boater interviews and boat inspections near Lake St. Clair, Michigan, and reported that most zebra mussels found on departing boats were attached to entangled macrophytes rather than directly attached to boats or equipment.

Zebra mussels can survive emersion for more than 10 days under favorable conditions (<15°C and high relative humidity) (McMahon *et al.* 1993), allowing them to be dispersed between bodies of water by trailered boats contaminated either directly with zebra mussels or with zebra-mussel laden, entangled macrophytes (Carlton 1993, McMahon *et al.* 1993, Kraft and Johnson 2000, Johnson *et al.* 2001). A few translocated adults can produce millions of microscopic veliger larvae (Stanczykowska *et al.* 1977), allowing rapid colonization of a new habitat (Johnson and Padilla 1996). Zebra mussels reach high population densities and transform entire lakes by removing planktonic algae, increasing the depth of the photic zone (Reeders and bij de Vaate 1990) and altering habitat structure for native benthic organisms (Griffiths 1993).

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Since their North American introduction in the mid 1980s, zebra mussels have invaded the Great Lakes; essentially the entire Mississippi River; portions of the Ohio, Tennessee, and Hudson Rivers; and are currently spreading west into portions of the Missouri and Arkansas River systems (Bensen and Boydstun 1995). As of the time of this writing (September 2004), zebra mussels have not yet established populations west of the 100th meridian, although there have been several reports of adult mussels found on boats as far west as California (Bensen and Boydstun 1995).

Predicting where zebra mussels may invade next is important for natural resource managers. Limited time and budgets necessitate focusing on key areas where outreach efforts will be most beneficial. However, attempts at predicting the overland dispersal of zebra mussels have had mixed results (Carlton 1993, Johnson and Carlton 1996, Johnson and Padilla 1996, Schneider *et al.* 1998, Buchan and Padilla 1999, Bossenbroek *et al.* 2001). Dynamics of the spread of invasive species are often studied with diffusion models that predict invasion by short-distance expansion of the periphery of established populations (Skellam 1951). Such models are often appropriate for terrestrial and marine invasions where suitable habitat is continuous; however, they are inappropriate for island systems or non-continuous freshwater systems where areas of suitable habitat may be separated by long distances. Many of the inland bodies of water near the Great Lakes were colonized by saltatory or "jump" dispersal rather than simple diffusion, leading to patchy colonization at regional scales (Kraft and Johnson 2000). Stratified diffusion models that account for both local diffusion and long-distance movements (Hengeveld 1989) have been used by some researchers to model the invasion of zebra mussel in North America (Buchan and Padilla 1999). Stratified diffusion models may be of some use for estimating the probability of invasion at various distances from source populations; however, these models are insufficient to identify specific bodies of water susceptible to invasion. Nevertheless, human-mediated dispersal vectors are discernable and play a key initial role in the invasion process (MacIsaac *et al.* 2001). By identifying opportunities for invasion (i.e., vectors), natural resource managers may potentially mitigate them, and thus slow or limit the spread of zebra mussels.

Gravity models are another approach for estimating where zebra mussels may spread. Bossenbroek *et al.* (2001) estimated the potential for invasion by zebra mussels in the northern midwest United States using gravity models that took into account estimates of the attractiveness of bodies of water to recreational boaters (based on surface area), the distances between locations, number of boaters per county, physical characteristics of potential destination lakes, and included a parameter for stochastic events. Stochastic gravity model predictions for the number of invaded lakes per county were fairly well correlated ($r^2 = 0.681$) with actual observations of sampled colonized lakes (Bossenbroek *et al.* 2001). Thus, gravity models appear promising when boater movement patterns cannot be estimated by expensive and time consuming boater surveys, which are frequently unavailable for areas of interest (Bossenbroek *et al.* 2001).

However, where resources are available, boater surveys can reveal true boater movement patterns on a more local scale than stratified diffusion and gravity models. For example, Buchan and Padilla (1999) analyzed boater movements in Wisconsin using just under 40,000 (useable) boater surveys conducted by the Wisconsin Department of Natural Resources (WDNR). Patterns of movement by recreational boaters based on the surveys were better at predicting patterns of invasion by zebra mussels than simple diffusion models. The six (as of 1999) inland lakes infested by zebra mussel in Wisconsin were found to be among the most frequently visited lakes (within the top 6.3%) according to WDNR surveys, and all of these lakes were linked by at least one trailered boat to the mussel-infested waters of Lake Michigan (Buchan and Padilla 1999). Thus, given adequate time and resources necessary to conduct them, boater surveys appear to be an effective tool for assessing the movements of recreational boaters and potential sites of future invasions by zebra mussels.

The Western Regional Panel of the Aquatic Nuisance Species Task Force recognized the importance of examining the movements of recreational boaters on a larger scale than has previously been attempted and assigned it as one of the primary objectives of the 100th Meridian Initiative. Under the guidance of the United States Fish and Wildlife Service, members of the 100th Meridian Initiative began conducting surveys in 1998 at popular boating locations in the U.S. states of Texas, Oklahoma, Kansas, Nebraska, South Dakota, and North Dakota, and the Canadian province of Manitoba, which straddle the 100th meridian. Surveys were later expanded to include bodies of water in Montana, Wyoming, Idaho, Oregon, and Washington, and most recently Lakes Powell and Mead in Utah, Arizona, and Nevada. Surveys were performed by various state, provincial, and regional agencies or organizations in order to assess the potential for spread of zebra mussels west of the 100th Meridian on trailered boats. Many of these surveys involved face-to-face interviews with boaters at boat launch sites, allowing collection of data on boaters' home zip codes and previous and future launch locations. Interviewees were also educated on the potential ramifications of spreading aquatic nuisance species and provided voluntary boat inspections to assess whether organisms (aquatic nuisance species or otherwise) were present on boats and associated equipment. Between

1998 and 2003, 100th Meridian Initiative surveyors found zebra mussels on only one inspected boat (a boat from Michigan visiting Sidney, Nebraska). However, other aquatic nuisance species, especially Eurasian water milfoil (*Myriophyllum spicatum*) and hydrilla (*Hydrilla verticillata*), were commonly discovered and removed from boats at numerous locations.

By December 2003 over 7,400 interviews had been accumulated in the 100th Meridian Initiative database stored at the Center for Biological Macrofouling Research at The University of Texas at Arlington. This database, updated as new data arrive, is available online at the 100th Meridian Initiative website (http://100thmeridian.org).

The specific aim of the current study was to use GIS techniques to analyze the 100th Meridian Initiative's boater-survey data accumulated from 1998 through 2002 in order to assess the traffic patterns of trailered boats. Resulting information was then used to determine which bodies of water near and west of the 100th meridian may be most at risk for receiving zebra mussels by way of "hitch-hiking" on trailered boats and to identify major highway segments best suited (based on traffic levels of trailered boats) for erecting signs or installing radio public-awareness message transmitters designed to educate boaters about the dangers of transporting zebra mussels to western states.

METHODS

Between 1998 and 2002 various state agencies and regional organizations conducted boater interviews for contribution to the 100th Meridian Initiative database. Boaters were typically interviewed at boat launch areas, but occasionally were interviewed at roadside rest areas on principal east-west highways that cross the 100th meridian. Surveys involved a standard form with a list of questions (Appendix 1). However, individual agencies were free to modify the survey form by adding or subtracting information according to their own needs. Only survey forms that included information regarding future and recent boater destinations were included in this study. Sample sites were chosen by the local agencies/organizations who conducted the surveys (Appendix 2). These agencies/organizations did not report how sampling sites were chosen. Individual agencies were also left to decide how to choose boaters for interviews. In some cases, all boaters during a given time period may have been interviewed, while in other cases, only some of the available boaters may have been interviewed. This information was not recorded. Boaters were not asked whether they had participated in the survey previously, so it is possible that some boaters may be represented in the dataset more than once.

All valid survey records entered in the 100th Meridian Initiative database from 1998 to 2002 were used in this study, including surveys from Texas, Oklahoma, Kansas, Nebraska, South Dakota, Wyoming, Montana, Idaho, Oregon, and Washington (Fig. 1). Named destinations from the 100th Meridian initiative were converted to geographic coordinates (latitude and longitude in decimal degrees) enabling the use of geographical information system (GIS) software to estimate the shortest possible route between a boater's origin and destinations using only major U.S. highways. These estimated routes are hereafter referred to as "trips" and were subsequently used to rank each segment of major U.S. highway by the amount of trailered boat traffic across it, and to determine which destinations were receiving the most trailered boat traffic from areas potentially infested with zebra mussels.

Converting 100th Meridian Initiative data

Raw data from the 100th Meridian Initiative included lists of names of bodies of water throughout North America, predominately in the states that straddle the 100th meridian and the northwest United States. Because data were collected by different volunteers, raw data included non-standardized geographic names. For example, a single body of water in Kansas was recorded in different database records as Lake Cheney, Cheney Lake, and Cheney Reservoir. All of the permutations for each body of water were searched by querying the United States Geological Survey's (USGS) Geographic

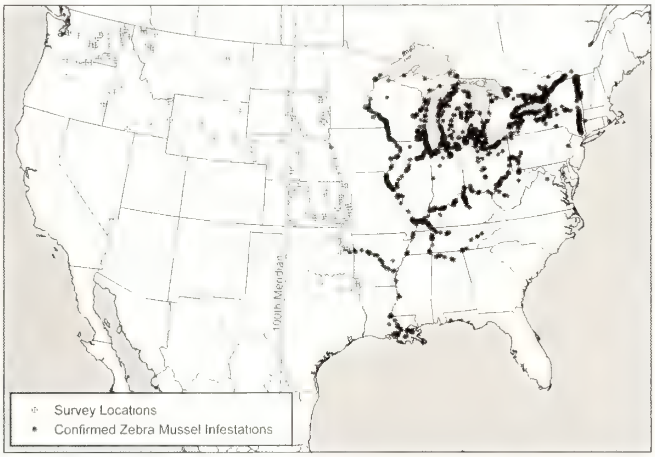

Figure 1. 100th Meridian Initiative boater survey locations from 1998 through 2002 (crossed circles) and the North American zebra mussel distribution through 2002 according to USGS records of confirmed zebra mussel sightings (asterisks). Since these data were compiled, new zebra mussel infestations have been confirmed in Virginia and Kansas, but these latest invasions were not included in the present study.

Names Information System (GNIS) and assigned geographic point estimates based on the official name according to USGS records (Table 1).

It was assumed that boaters began each trip from their homes. It is important to note that simply returning home with one's boat after visiting a potentially contaminated lake does not reduce the probability of transporting live zebra mussels to zero. On the contrary, zebra mussels can survive for several days out of water (McMahon *et al.* 1993) and may be able to survive even longer in wet bilge areas, live-bait wells, bait buckets, and other areas that retain water. It is reasonable to assume that boaters returned home between visits to different bodies of water. Thus, point data representing home zip code area centroids were used as estimates for boaters' origins. A GIS layer of U.S. zip code centroids was obtained from the National Oceanographic and Atmospheric Administration (NOAA) (Table 1). Coordinates of latitude and longitude for zip code centroids were joined to corresponding records in the 100th Meridian Initiative database.

100th Meridian Initiative data

Data tables from the 100th Meridian Initiative database in Microsoft Access (version 9.0) format were exported to dBase IV format (.dbf) and then imported into ArcView GIS software (Environmental Systems Research Institute, Inc. [ESRI], Redlands, California). From these tables, origin coordinates (geographic points), hereafter referred to as "origins" were added as an "event theme" and saved as a new GIS layer (shape file). Destination coordinates, hereafter referred to as "destinations" were also imported in the same manner. Survey locations, a subset of destinations corresponding to locations where interviews were conducted, were added as a third GIS layer. Since origins and destinations were imported from the same table, attribute data for both were identical and included a unique record (ID) number corresponding to an individual "Trip." Thus, each origin was associated with each destination by their shared ID number. It was necessary to create two shape files from the same table (one for origins and one for destinations) in order to accommodate the requirements of the program-

Table 1. Sources for GIS Layers

Description	Source	Date	Features
Zip code centroids for United States	National Oceanographic and Atmospheric Administration URL: http://cads.nos.noaa.gov File name: us_zipcode_points.shp	1999	Points
U.S. States	ESRI data and maps CD File name: states.shp	2002	Polygons
Canadian Provinces	ESRI data and maps CD File name: provinces.shp	2002	Polygons
North American distribution of confirmed zebra mussel infestations	United States Geological Surgery, Florida Caribben Science Center URL: http://nationalatlas.gov File name: zmusslex020.shp	2001	Points
Major U.S. roads	United States Geological Survey URL: http://nationalatlas.gov File name: roadtrl020.shp	2002	Lines
Streams and bodies of water of the U.S.	United States Geological Survey URL: http://nationalatlas.gov File name: hydrogm020.shp	2003	Polygons
100th Meridian Initiative boater interview locations	100th Meridian Initiative Database standardized by USGS Geographic Names Information System URL: http://100thmeridian.org File name: surveys.shp	2003	Points
100th Meridian Initiative boater origins	100th Meridian Initiative Database (see Methods) URL: http://100thmeridian.org File name: trips_orig.shp	2003	Points
100th Meridian Initiative boater destinations	100th Meridian Initiative Database (see Methods) URL: http://100thmeridian.org File name: trips_dest.shp	2003	Points

ming code (in Avenue scripting language) written to automate the network analysis process (described later).

Additional data

In order to facilitate estimates of shortest-routes from each origin to destination, a GIS layer (line data) of major U.S. highway segments (including Interstates and federal and state highways) was obtained from the USGS (Table 1). A GIS layer containing the distribution of infestations by zebra mussel through 2001 was also obtained from the USGS (Table 1) and added as points. Additional baseline (polygon) data were also added for geographic reference, including two GIS layers representing the conterminous United States and Canadian provinces from ESRI's supplemental Data and Maps CD (Table1). All GIS layers were converted to the North American Datum 1983 reference system.

At-risk destinations

ArcView 3.2 was used to select all boaters who listed a destination within a 40-km radius of any confirmed zebra mussel infestation, based on the data on the distribution of zebra mussels obtained from the USGS. Buchan and Padilla (1999) reported that 91.6% of surveyed boaters traveled less than 50 km with a mean of 34 km. Based on these figures we chose 40 km as a reasonable estimate of how far most boaters might travel. Boats visiting destinations within 40 km of confirmed sightings of zebra mussels were considered for this study as potentially contaminated. Using only records for boaters with potentially contaminated boats, all additional destinations (those >40 km from known zebra mussel infestations) were then selected. These currently non-infested destinations were considered potentially at risk of receiving zebra mussels because they were visited by potentially contaminated boats. These "at-risk" destinations were then ranked by the number of trips to each destination made by boaters known to have also visited areas within 40 km of infestations of zebra mussels (Table 2). The assumption was made that bodies of water receiving a larger number of trips by boaters that recently launched in zebra-mussel infested areas are more likely to be invaded by zebra mussels than bodies of water receiving fewer or no trips from these areas. At each of the top ten destinations the proportions of boats recently launched in infested areas relative to non-infested areas were compared. A chi square analysis was used to test whether an equal proportion of "potentially contaminated boats" were being launched at the most visited (top ten) non-infested sites by boaters coming from areas near infestations of zebra mussels. The null hypothesis was that there was an equivalent proportion of boaters coming from areas infested by zebra-mussel launching at each of these sites. Rejection of the null hypothesis would suggest that some non-infested sites are more likely than others to receive zebra mussels via trailered boats.

Estimation of routes from origins to destinations

ArcView version 3.2 and the Network Analyst extension (ESRI, Redlands, California) were used to estimate the shortest routes along major U.S. highways between each origin and destination pair in this part of the analysis. A boat was assumed to be potentially contaminated if the boater listed at least one destination within 40 km of any known infestation of zebra mussels. Only trips made by potentially contami-

Table 2. Top ten-at-risk destinations ranked by the number of trips to each destination made by surveyed boaters who said that they also visited areas within 40 km of sites of infestations by zebra mussels. Total trips are the total number of trips made by all surveyed boaters to the corresponding location regardless of where they had been. Observed risky trips are the number of trips made by potentially contaminated boats. Expected risky trips are the number of trips expected at each location based on the (rejected) null hypothesis that the frequency of boats coming from areas with known infestations by zebra mussels is equal across all of the ten listed destinations.

Rank	Location	State	Total trips	Observed risky trips	Expected risky trips†	χ^2
1	Lake Francis Case	SD	2572	44	90	23.692*
2	Cheney Reservoir	KS	655	33	21	6.182
3	El Dorado Lake	KS	549	22	18	0.888
4	Table Rock Lake	MO	170	16	6	19.500
5	Winfield City Lake	KS	328	15	11	1.675
6	Lake Fork Reservoir	TX	33	13	1	131.265*
7	Grand Lake o' the Cherokees	OK	142	11	5	8.643
8	Lake Oahe Dam	SD	646	10	21	5.903
9	Lewis & Clark Lake	SD	223	9	7	0.390
10	Keystone Lake	OK	22	8	1	73.441*

* $p \leq 0.005$ ($\alpha = 0.005$ for Bonferroni adjustment); $\chi^2_{crit} = 23.59$ (df = 9).

† Expected numbers are rounded to the nearest whole number.

nated boats were used. Network Analyst's built-in function can only calculate the shortest route for a single origin/destination pair without automation via programming code. An Avenue script (written by Dan Patterson, Department of Geography and Environmental Studies, Carleton University) allowed automation of this process for multiple origin/destination pairs. This script, obtained from ESRI, required modification because it was designed to calculate the shortest paths between *all* possible combinations of multiple origins and destinations, but only specific combinations were necessary. Thus, Patterson's script was modified to compute paths only for the selected origin/destination pairs in the 100th Meridian Initiative record set. The executed script generated a new GIS layer (line data) containing the estimated shortest route following major highways between each origin (home zip code centroid) and destination (body of water) for each trip made by a boater who listed at least one destination within 40 km of a known site of infestation by zebra mussels. Each route represented an individual trip from a boater's home to his or her destination.

Traffic levels across specific highway segments

The Major U.S. Roads GIS layer (Table 1) contained highways broken into segments. In this context "segment" is defined as a stretch of highway starting at one intersection and ending at the next. Thus, individual highways in the Major U.S. Roads GIS layer comprised numerous (46,014) segments. A second ArcView extension (also written by Dan Patterson) was used to count the number of times that trailered boats crossed each segment of highway from origin to destination. Patterson's extension was designed to automate ArcView's Select-by-Theme function by selecting and counting the number of target features that have a specific geographical relationship with each selector feature. The extension then appends the count for each selector feature to the selector layer's attribute table. For this study, the Major U.S. Roads GIS layer (of highway segments) was used as the selector layer and the GIS layer containing the shortest-routes (or trips) calculated earlier was used as the target layer. When executed, the extension selected and counted all "trips" that contained the center of each highway segment in the Major U.S. Roads GIS layer and added the corresponding count to each highway segment record in the GIS layer's attribute table.

RESULTS

At-risk destinations

The database of surveys of the 100th Meridian Initiative conducted between 1998 and 2002 (Fig. 1) includes data from 7,412 boaters listing a combined total of 793 unique destinations (Fig. 2) in 20,639 records. Each of these records represents an individual "trip" made by a boater hauling a boat from one location to another. If a boater listed a destination within 40 km of a confirmed site of infestation by zebra mussel, based on USGS data on the distribution of zebra mussels (Fig. 1), the corresponding boat was considered to be potentially contaminated. Thus, all other destinations visited by such a boat are possibly at risk of invasion by "hitch-hiking" zebra mussels. A total of 160 trips to 48 unique destinations within 40 km of confirmed infestations were found to be made by surveyed boaters (Fig. 3). These destinations were potential sources of contamination for boats transported elsewhere. The distribution of distances traveled by boats coming from infested areas ("risky boats") and from non-infested areas ("non-risky boats") is shown in Fig. 4.

A total of 93 non-infested bodies of water in 16 states and one Canadian province were found to be visited by potentially contaminated boats (Fig. 3). Kansas had more at-risk destinations than any other state: 26 bodies of water in Kansas were visited a total of 140 times by potentially contaminated boats, with Cheney Reservoir, El Dorado Lake, and Winfield City Lake accounting for half (70) of these trips. Based solely on number of visits by potentially contaminated boats, Lake Francis Case in South Dakota and Cheney Reservoir and El Dorado Lake in Kansas (with 44, 33, and 22 visits, respectively) were found to be the bodies of water most at risk of receiving hitch-hiking zebra mussels. After Kansas, South Dakota was the second most-visited state with a total of 71 visits by potentially contaminated boats. The geographic distribution of all at-risk destinations is shown in Fig. 3.

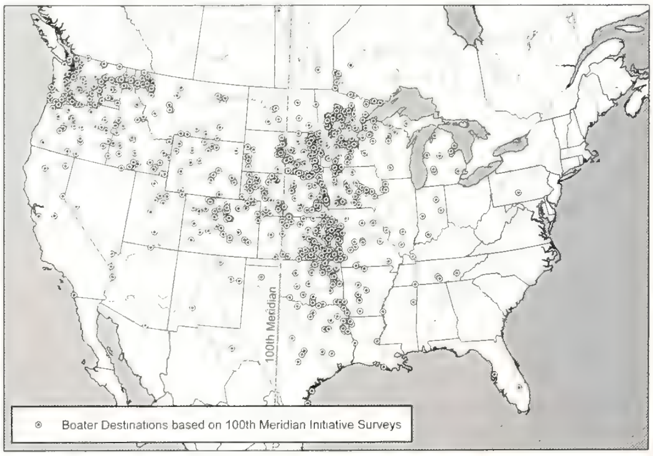
100th Meridian
⊚ Boater Destinations based on 100th Meridian Initiative Surveys

Figure 2. Water body destinations (dotted circles) as reported by boaters surveyed by participants of the 100th Meridian Initiative between 1998 and 2002.

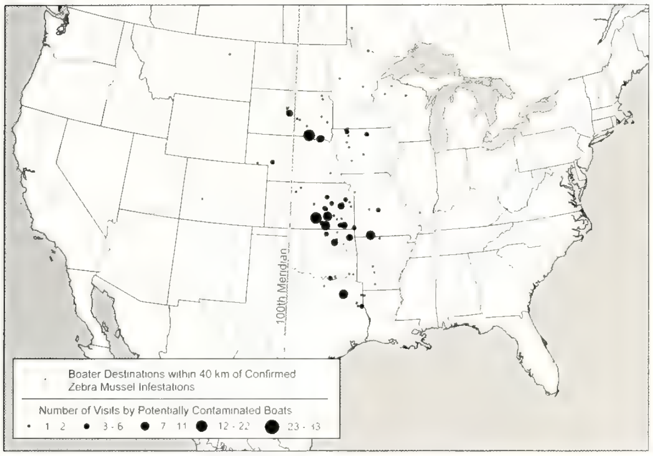
100th Meridian
Boater Destinations within 40 km of Confirmed Zebra Mussel Infestations
Number of Visits by Potentially Contaminated Boats
1 2
3 - 6
7 11
12 - 22
23 - 43

Figure 3. Boater destinations (dotted circles) within 40 km of confirmed zebra mussel sightings (according to USGS data) and non-infested bodies of water (black circles) ranked by symbol size according to the number of visits made by potentially contaminated boats. Data are from 100th Meridian Initiative surveys conducted between 1998 and 2002. Potentially contaminated boats are those reported to have visited a water body within 40 km of a confirmed zebra mussel sighting during the survey period.

The top ten at-risk destinations (ranked by total number of visits by potentially contaminated boats) were analyzed to assess whether each was equally likely of receiving potentially contaminated boats. This null hypothesis was rejected ($\chi^2 = 280.8$; df = 9; $p < 0.001$) in favor of the alternative hypothesis that some locations were receiving potentially contaminated boats at higher rates than others. Lake Francis Case and Oahe Dam in South Dakota both had several visits by potentially contaminated boats (ranked numbers 1 and 8, respectively), but less so than would be expected if all of the top ten destinations were visited at equal rates. These differences between observed and expected frequencies were statistically significant for Lake Francis Case but not for Oahe Dam after Bonferroni adjustment ($\alpha = 0.005$) to account for experiment-wide error (Table 2). Two locations had significantly more visits by potentially contaminated boats than expected (Table 2): Lake Fork in Texas and Keystone Lake in Oklahoma respectively accounted for only 0.16% and 0.11%, respectively, of the destinations listed by all surveyed boaters, yet accounted for 8.1% and 5.0%, respectively, of the destinations visited by potentially contaminated boats.

Traffic levels across specific highway segments

The major U.S. roads utilized by surveyed boaters traveling between areas within 40 km of confirmed zebra-mussel sightings and non-infested areas are depicted with the most frequently used segments highlighted in Fig. 5. The network of highways utilized by potentially contaminated boats reached from infested areas to northern California, southwest Colorado, eastern Montana, central Florida, and all the states bisected by the 100th Meridian. Some of the longest trips made by surveyed boaters included Interstate 80 linking northern California to areas infested by zebra mussels east of the 100th Meridian, Interstate Highway 94 (I-94) linking Montana to Minnesota, and I-24 and I-75 from Tennessee through Georgia and south into Florida.

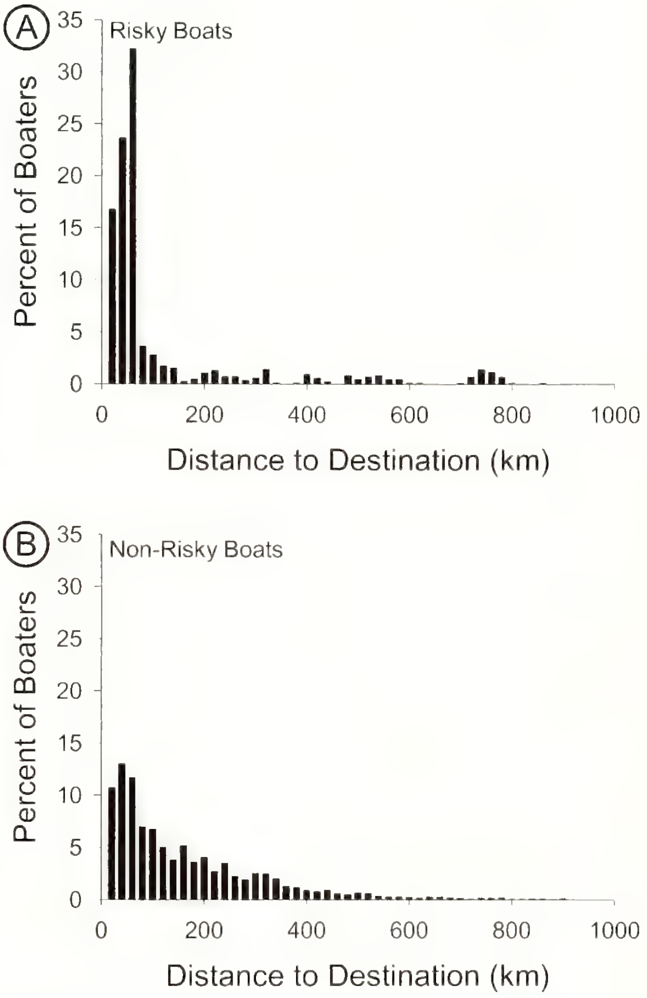

Figure 4. Distribution of the percent of boaters traveling various distances from their origin. Origins were assumed to be the centroid of the boater's home zip code area. Destinations are those reported by the boater. "Risky boats" are those who listed a recent destination within 40 km of a confirmed zebra mussel infestation. "Non-risky boats" are those who did not list a destination near zebra mussel infestations.

Three interstate highways (I-70, I-80, and I-94) were found to be traveled by boaters hauling potentially contami-

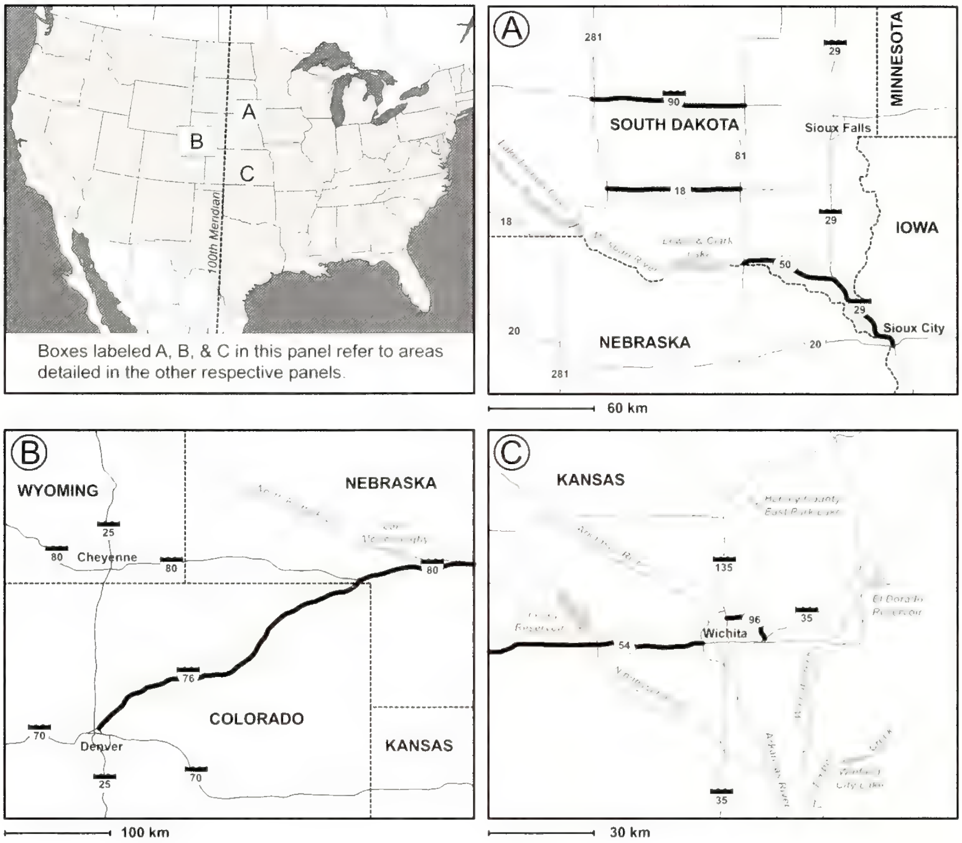
A
B
C
100th Meridian
Boxes labeled A, B, & C in this panel refer to areas detailed in the other respective panels.
A
281
90
29
MINNESOTA
SOUTH DAKOTA
Sioux Falls
81
18
18
29
IOWA
Lewis & Clark Lake
50
29
Sioux City
20
NEBRASKA
20
281
60 km
B
WYOMING
NEBRASKA
25
80
Cheyenne
80
80
76
70
COLORADO
Denver
25
70
KANSAS
100 km
C
KANSAS
135
96
35
Wichita
54
El Dorado Reservoir
Arkansas River
35
30 km

Figure 5. Major U.S. highway segments most utilized for overland transport of potentially contaminated boats according to boater survey data collected between 1998 and 2002. The first panel provides a general location reference for areas detailed in the other panels labeled A, B, and C. Roads that carried >500 potentially contaminated boats are highlighted in panels A, B, and C with thick black lines. Potentially contaminated boats are those reported to have visited a water body within 40 km of a confirmed zebra mussel sighting during the survey period. Highway segments indicated here are well suited for public outreach efforts designed to slow or prevent the spread of aquatic nuisance species into western North America.

nated boats across the 100th Meridian into western states. The roads most heavily used were stretches of I-90 and I-29, U.S. Highway 18, and State Highway 50 in southwest South Dakota (Fig. 5A); I-76 in northeast Colorado continuing into I-80 in southwest Nebraska (Fig. 5B); and State Highway 96 and U.S. Highway 54 in Kansas (Fig. 5C), each carrying over 500 potentially contaminated boats during the survey period.

DISCUSSION

The data in this analysis are clearly biased in favor of areas where most surveys were performed. Specifically, Kansas, Nebraska, and South Dakota were better represented because these states contributed a large proportion of the data. Areas where few or no surveys were conducted (e.g., most of the southwest U.S.) were not well represented. However, some boaters from surveyed states listed destinations in non-surveyed states. Thus, this information should be useful to state agencies and public organizations in areas not well represented in the analysis. In particular, southwestern states and California should consider conducting boater surveys for contribution to the 100th Meridian Initiative database in order to prevent under-representation in future analyses.

Inherent bias in this analysis reduced the ability to detect at-risk areas in under-sampled regions (i.e., the American Southwest). However, the at-risk nature of locations in highly sampled areas (i.e., Kansas, South Dakota, and Nebraska) is much less tenuous. This study was especially useful for highlighting at-risk areas near the heavily surveyed locations near the 100th meridian. Presumably, local agencies selected survey sites based on their concern for receiving boats contaminated with zebra mussels and other aquatic nuisance species. Thus, areas sampled more than others probably correspond to areas where concern is greatest. Therefore, agencies in areas generating the most survey contributions are the ones that will benefit most from this analysis of boater movements.

Buchan and Padilla (1999) reported that frequent long-distance dispersal paths are rare, but should be identified for focusing management efforts. The current study found that many boaters traveled to or from destinations outside of surveyed areas. Although poorly sampled, these areas are not excluded from being highlighted as potentially at risk. For example, Table Rock Lake in southern Missouri was the fourth most-visited lake by potentially contaminated boats despite there being no surveys from Missouri in the 100th Meridian Initiative database. Connections between non-surveyed states and areas harboring zebra mussels should not be ignored. For example, Blue Mesa Reservoir in Colorado, a state not surveyed, received two visits from a boater who had recently visited infested areas. One boater from Elk Grove, California, reported taking his boat thousands of kilometers to an infested area in Michigan. Although it is possible that such trips represent rare events, boater surveys reveal they are nonetheless occurring. Because of the ability of zebra mussels to rapidly colonize a new habitat, it is possible that a single translocation of zebra mussels could place a western body of water in jeopardy of invasion. Thus, authorities in western locations linked to infested areas by overland recreational boat transport should be concerned with even single visits from boaters from waters with zebra mussels.

Although most 100th Meridian Initiative boater surveys were not conducted in areas where sightings of zebra mussel have been confirmed (with the notable exception of a single area on the Missouri River bordering Nebraska, about 15 miles south of Sioux City, Iowa), several highways outside of these areas are being used for overland transport of potentially contaminated boats. Our analyses allowed for a visual representation of bodies of water potentially most at risk of introduction of zebra mussels via trailered boat traffic and highlighted the highway segments potentially most used by surveyed boaters who visited areas known to harbor zebra mussels. Although most surveyed boaters said that they usually launch their boats in areas near their homes, there were several instances in which boaters transported boats hundreds of kilometers west to distant bodies of water. Essentially any area in the conterminous U.S. can be reached by motor vehicles within the tolerated emersion times of zebra mussels under favorable conditions (high humidity and moderate temperature; McMahon *et al.* 1993).

Although the interstate highways reaching as far as California, Colorado, Montana, and Florida were not among the most traveled by surveyed boaters, they are important because they highlight how far recreational boaters may travel during a single trip. Thus, authorities and managers interested in preventing invasion of bodies of water by zebra mussels and other non-indigenous aquatic species throughout North America should be aware of the potential for their dispersal by overland boat transport and should educate the boating public on taking appropriate precautions to prevent their acting as dispersal vectors for these species. The bodies of water receiving trailered boats from areas known to be infested with zebra mussels are illustrated in Figure 3. Local authorities for these bodies of water, especially those receiving the highest levels of traffic from infested areas (Table 2), should monitor their jurisdictions closely and educate boaters about zebra mussels and other aquatic nuisance species in order to help prevent invasions of these waters.

Several highway segments were identified as parts of the shortest routes between boaters' origins and destinations. Notable highway segments carrying the most boaters known to be visiting infested areas were U.S. Highway 18, State Highway 50, and I-90 in South Dakota east of Lake Francis Case (Fig. 5A); I-80 in Nebraska south of Lake McConaughy connecting with I-76 in Colorado running by Prewitt and Empire Reservoirs (Fig. 5B); U.S. Highway 54 in Kansas south of Cheney Reservoir; and State Highway 96 in Kansas west of El Dorado Lake (Fig. 5C). The high level of trailered boat traffic in these highway segments make them well suited for public outreach efforts such as installations of Traveler Information Systems (TIS), warning against the transport of aquatic nuisance species. TISs are short-range radio transmitters that broadcast public service announcements near highways. Signs accompany these transmitters to inform motorists of the specific radio frequency used for the broadcast. Such TIS transmitters informing boaters of the risk of overland transport of zebra mussels and other aquatic nuisance species have already been installed near highways in Montana by Montana Fish, Wildlife, and Parks in preparation for the Lewis and Clark Bicentennial Celebration and in northeast Oklahoma by the 100th Meridian Initiative.

Since the analysis presented herein was completed, zebra mussels have been confirmed in four areas identified to be "at risk" by the study. The Kansas Parks and Wildlife Department announced on 25 August 2003 confirmation of an established infestation by zebra mussel in El Dorado Lake in south central Kansas near the 100th meridian (~96.8°W longitude). El Dorado Lake was ranked in this study as the third most-visited water body by potentially contaminated boats. Segments of U.S. Highway 54 and State Highway 96 in south central Kansas were also highlighted here as among the most traveled by boaters trailering potentially contaminated boats. Based on shell sizes and population densities, it was estimated that zebra mussels had been in El Dorado Lake for two or more years (T. Mosher pers. comm.). Kansas is now the second state straddling the 100th Meridian to become invaded by zebra mussels (Oklahoma was the first). Later in 2003, veligers of zebra mussel were confirmed in the Missouri River bordering Nebraska and South Dakota downstream of Fort Randall Dam on Lake Francis Case (~98.0°W longitude) and Gavin's Point Dam on Lewis & Clark Lake (~97.2°W longitude). Because veliger larvae are planktonic, adult populations must have established upstream of these sampled locations, probably in the lakes above the dams. In August 2004, veligers were also confirmed in Cheney Reservoir on the North Fork of the Ninnescah River in south central Kansas (~97.8°W longitude). These most recent invasions emphasize the utility of boater survey data obtained as part of the 100th Meridian Initiative and GIS analyses as performed for this study to evaluate the most susceptible bodies of water and most likely routes of zebra mussel invasion in the western U.S. Meanwhile, zebra mussels continue to spread west and may soon become established beyond the 100th meridian.

Preventing the spread of aquatic nuisance species is an important endeavor essential for the conservation of native species and freshwater habitats everywhere. This study focuses on identifying opportunities for dispersal of zebra mussels by examining links between areas already infested and those not yet invaded. Preventing further spread may best be achieved by targeting public outreach and monitoring efforts in the popular boating areas identified in this study. It is hoped that this study will help to raise awareness of the potential to disperse zebra mussels by overland boat transport in the western U.S. and to encourage action by authorities and managers of western bodies of water, particularly those identified in this report as being most potentially at risk of introduction.

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Appendix 1. Survey questions.

Question	Description
Interviewer	Name of the person conducting the survey
Date/Time	Time and date of the survey
Body of water	Name of the location where the interview was conducted including the state
Launch Site	Any more specific information regarding the location of the survey
Survey type	Either "contact" if an interview was conducted or "observation" if the boater was merely observed
Home State	Interviewed boater's home state
Zip Code	Interviewed boater's home zip code
Type of Transport	"Commercial use" or "personal use"
How many times have you launched in the last year?	Number of launches
Do you always launch in the same body of water?	Yes or no
Type of boat	Angling, pleasure, sail, jon, canoe, etc.
Where else have you launched recently? Space was provided for up to three locations.	Boaters were asked to provide the name of the body of water, the state, the county, and the date of the launch (if known).
Where do you plan to launch next? Space was provided for up to two locations.	Boaters were asked to provide the name of the body of water, the state, the county, and the date of the launch (if known).
Do you clean your boat and trailer between launching?	Yes or no
Is your boat kept in water or on land when not in use?	Water or land
Did the surveyor give the boater any literature?	Yes or no
Did the boater ask questions?	Yes or no
Inspection	Rejected or conducted
Inspection Results, anything found?	Yes or no
Inspection Conducted by?	Interviewer, boater, or both
What was found?	List of any aquatic nuisance species found
Where was it found?	*e.g.*, "on boat motor"
What actions were taken?	*e.g.*, "disposed of in trash"
Comments	Anything the surveyor or boater wishes to add.

Appendix 2. Number of surveys at each location

Body of water	State/Province	Number of surveys
American Falls Reservoir	Idaho	5
Brownlee Reservoir	Idaho	7
Coeur d'Alene	Idaho	24
Crane Creek Reservoir	Idaho	1
Henry Lake	Idaho	14
Island Park Reservoir	Idaho	8
Lucky Peak	Idaho	16
Palisades Lake	Idaho	18
Payette Lake	Idaho	4
Pend Oreille	Idaho	34
Priest Lake	Idaho	16
Salmon Falls Creek Reservoir	Idaho	2
Turner Gulch	Idaho	6
Atchison State Fishing Lake	Kansas	3
Augusta Lake	Kansas	1
Banner Creek Reservoir	Kansas	5
Belle Plaine Visitor Center	Kansas	292
Big Hill Lake	Kansas	105
Brown State Lake	Kansas	1
Butler County State Lake	Kansas	13
Butler State Fishing Lake	Kansas	6
Cedar Bluff Reservoir	Kansas	38
Cheney Reservoir	Kansas	241
Clark County State Lake	Kansas	4
Clinton Lake	Kansas	123
Coffey County Lake	Kansas	2
Coldwater Lake	Kansas	10
Council Grove Lake	Kansas	8
Cowley County State Lake	Kansas	21
Crawford Lake	Kansas	8
El Dorado Lake	Kansas	154
Elk City Lake	Kansas	59
Empire Lake	Kansas	8
Fall River Lake	Kansas	10
Ft. Scott Lake	Kansas	11
Glen Elder Reservoir	Kansas	10
Hillsdale Lake	Kansas	91
John Redmond Reservoir	Kansas	6
Kanopolis Lake	Kansas	33
Keith Sebelius Lake	Kansas	2
Kirwin Reservoir	Kansas	19
La Cygne Lake	Kansas	12
Lake Afton	Kansas	50
Lake Parsons	Kansas	5
Lovewell Reservoir	Kansas	29
Marion Reservoir	Kansas	125
Meade State Fishing Lake	Kansas	2
Melvern Lake	Kansas	93
Milford Lake	Kansas	33
Mined Lakes	Kansas	2
Mission Lake	Kansas	3

Appendix 2. (continued)

Body of water	State/Province	Number of surveys
Mound City Lake	Kansas	2
Neosho State Fishing Lake	Kansas	5
Norton County Lake	Kansas	2
Norton Lake	Kansas	5
Parsons Lake	Kansas	7
Perry Lake	Kanses	13
Pomona Lake	Kansas	36
Rest Stop	Kansas	1
Toronto Lake	Kansas	4
Tuttle Creek Lake	Kansas	17
Waconda Lake	Kansas	25
Webster Lake	Kansas	40
Wellington Lake	Kansas	13
Wilson Lake	Kansas	58
Winfield City Lake	Kansas	117
Wolf Creek Reservoir	Kansas	11
Woodson State Fishing Lake	Kansas	1
Wyandotte County Lake	Kansas	4
Emerson	Manitoba	108
South Junction	Manitoba	36
Sprague	Manitoba	218
Bull Lake	Montana	8
Flathead Lake	Montana	16
Fort Peck Lake	Montana	148
Koocanusa	Montana	15
Lake Mary Ronan	Montana	24
Mariner's Haven Campground	Montana	2
Peck Gulch	Montana	1
Rexford Bench	Montana	36
Somers Reservoir	Montana	78
Sophie Lake	Montana	1
Trout Creek	Montana	3
Wayfarer—Flathead Lake State Park	Montana	143
Whitefish Lake	Montana	1
Blair Optimist Park	Nebraska	16
Box Butte Reservoir	Nebraska	141
Cabelas Wildlife Display	Nebraska	8
Calamus Reservoir	Nebraska	146
Davis Creek Reservoir	Nebraska	1
Elwood Reservoir	Nebraska	70
Harlan County Reservoir	Nebraska	100
Haworth Park	Nebraska	29
Johnson Lake	Nebraska	4
Lake McConaughy	Nebraska	421
Lake Minatare	Nebraska	147
Medicine Creek Reservoir	Nebraska	6
Merritt Reservoir	Nebraska	112
Missouri River	Nebraska	271
NP Dodge Park Campground	Nebraska	9
Red Willow Reservoir	Nebraska	20
River View Marina	Nebraska	24
Schilling WMA	Nebraska	4
Sherman Reservoir	Nebraska	57
Sidney East Reservoir	Nebraska	21
Chouteau Lock and Dam	Oklahoma	8
Eufaula Lake	Oklahoma	1
Fort Gibson Lake	Oklahoma	23
Keystone Lake	Oklahoma	6
McClellan-Kerr Arkansas River Navigation System	Oklahoma	1
Newt Graham Lock and Dam	Oklahoma	7
Oologah Lake	Oklahoma	1
Tenkiller Lake	Oklahoma	2
Billy Chinook	Oregon	16
Boardman Park	Oregon	26
Brownlee Reservoir	Oregon	14
Columbia River	Oregon	8
Dalles Boat Launch	Oregon	1
John Day River	Oregon	82
Lake Owyhee	Oregon	18
Bad River	South Dakota	7
Bitter Lake	South Dakota	18
Brant Lake	South Dakota	26
Cattail Lake	South Dakota	68
Chamberlain Recreation Area	South Dakota	15
Enemy Swim Lake	South Dakota	67
Fort Randall Dam	South Dakota	23
Fort Randall Tailwaters	South Dakota	80
Francis Case	South Dakota	4
Herman Lake	South Dakota	2
Joe Creek	South Dakota	3
Lake Brant	South Dakota	4
Lake Francis Case	South Dakota	765
Lake Madison	South Dakota	3
Lake Oahe	South Dakota	180
Lake Poinsett	South Dakota	18
Lake Sharpe	South Dakota	407
Lake Thompson	South Dakota	25
Lewis & Clark Lake	South Dakota	174
Little Bend	South Dakota	2
Lynn Lake	South Dakota	6
Madison Lake	South Dakota	13
Poinsett Lake	South Dakota	2
Rest Stop I-90	South Dakota	54
Thompson Lake	South Dakota	5
Waubay Lake	South Dakota	84
West Bend	South Dakota	5
Caddo Lake	Texas	2
I-30 Rest Stop (Texarkana)	Texas	14

Appendix 2. (continued)

Body of water	State/Province	Number of surveys
Lake Fork Reservoir	Texas	14
Lake O' the Pines	Texas	1
Lake Texoma	Texas	15
Ray Roberts Lake	Texas	1
Wright Patman Lake	Texas	2
Banks Lake	Washington	20
Central Ferry State Park	Washington	7
Chief Timothy State Park	Washington	9
Clarkston Boat Launch	Washington	4
Crow Butte State Park	Washington	20
Diamond Lake	Washington	25
Drano Lake	Washington	85
Fish Hook	Washington	4
Lake Cascade Park	Washington	1
Lake Roosevelt	Washington	1
Liberty Lake	Washington	24
Loon Lake	Washington	2
Lyons Ferry Marina	Washington	1
Lyon's Ferry State Park	Washington	7
Moses Lake	Washington	33
Plymouth Park	Washington	22
Pot Holes State Park	Washington	34
Snake River	Washington	41
Sprague Lake	Washington	3
Spring Canyon	Washington	30
Steam Boat Rock State Park	Washington	7
Wind River	Washington	32
Boysen Reservoir	Wyoming	33
Flaming Gorge	Wyoming	15
Glendo Reservoir	Wyoming	14
Keyhold Reservoir	Wyoming	2
Pathfinder Reservoir	Wyoming	14

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BOOK REVIEW

Taming of the Oyster: A History of Evolving Shellfisheries and the National Shellfisheries Association by Melbourne R. Carriker (2004). Sheridan Press, Hanover, Pennsylvania. 264 pp. ISBN: 0-9752881-0-5

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Long-time American Malacological Society member and past-president (1985) Melbourne R. Carriker has produced a carefully documented archival history of how the American shellfisheries industry organized itself and how its relationships with relevant government agencies, laboratories, and biologists evolved. The book focuses mainly on the people who developed the industry in the northeast United States, and especially on how they joined in associations for its betterment. The author, now in his ninetieth year and professor emeritus of marine biology and biochemistry at the University of Delaware, presents this institutional history as no one else could or would in a labor of love. The book valuably documents and preserves in print for posterity information of a type all too often lost or relegated to the proverbial dustbin of history.

If you are a malacologist with interests distant from commercial aspects, however, the book may tell you more than you want to know about the National Shellfisheries Association (NSA) and its antecedents over the last 95 years. Carriker frequently alludes to applied laboratory research on oysters, but biological aspects are usually listed briefly or discussed only minimally. In much of the book the biology of the oyster itself remains largely offstage. Its subtitle thus better encapsulates the content of the book than the title.

I was surprised to learn that the U.S. commercial oyster shucking, packing, and shipping industry was born in the 1820s-1830s in New Haven, Connecticut (where I was also born, a century later) and that the first trade group, the Oyster Growers and Dealers Association (OGDA), was organized by the major New Haven oyster grower Henry C. Rowe in 1908. Even earlier, however, biologists had begun to study the U.S. oyster industry, particularly three illustrious students of Louis Agassiz at Harvard. These were Addison E. Verrill (Verrill and Smith 1874) of Yale, Ernest Ingersoll (1881), and W. K. Brooks (1891) of Johns Hopkins. More than a century ago, they observed and clearly articulated threats to the industry from overharvesting and from habitat degradation due to pollution. As Carriker documents, our modern concerns have a long history.

The next generation of biologists interested in oysters and oystering comprised students of Brooks, who was the first American professor to train Ph.D. students in biology. Carriker lists a dozen of these who were active in shellfisheries biology in the first half of the 20th Century, including Verrill, Robert E. Coker, Gilman Drew, Caswell Grave, J. L. Kellogg, and Julius Nelson. Early in the century, some of these biologists addressed and worked with a new organization, the National Association of Shellfish Commissioners (NASC), founded in 1909 and representing the eastern seaboard states. Carriker weaves together the development and relationships of the OGDA and NASC—the latter broadening its mission to include fin and crustacean fisheries from 1915 as the National Association of Fisheries Commissioners (NAFC)—and finally morphing into the NSA in 1930.

Carriker's account of the period between World Wars I and II especially attracted my personal interest, because much of the most successful applied oyster research of the time occurred at Milford, Connecticut, where I grew up from 1934 to 1941. I remember well the roads near the shore paved with crushed and rolled oyster shells. Carriker includes a detailed account and several photos of the evolution of the Milford Laboratory from a modest structure donated by the Connecticut Oyster Farms Company to a well-established federal laboratory in an attractive Georgian revival building. This laboratory, and the era, was dominated by Victor Loosanoff, who prefaced his public lectures to Milford townspeople with, "As you know I am interested from oysters . . ." Loosanoff, a White Russian military officer, escaped from the Bolsheviks across Siberia, China, and Japan and wound up working in a lumber camp in Washington State. As Carriker well describes him, Loosanoff was "physically strong and large of stature with broad shoulders and bulging biceps" (p. 44). Carriker does not mention that Loosanoff, discovered by a coach, was recruited to play football at the University of Washington. There, influenced by Professor Trevor Kincaid, he also became a serious student of marine biology. After graduating in 1927, Loosanoff took a position as Chief Marine Biologist of Virginia, then began both work at the Milford Laboratory and graduate study at Yale a year later. Loosanoff completed his Ph.D. in 1941, and under his direction the Milford Laboratory became "the birthplace [of] the early techniques used by shellfish hatcheries in the United States" (Chew 2002).

Biological studies that practical oystermen could use to improve their business practices began to emerge as an important component of the NAFC and OGDA at their joint meetings starting in 1928. These studies focused mainly on oyster nutrition and propagation, and on water quality and other aspects of sanitation. Carriker quotes the *Fishing Gazette* account of the 1929 meeting, that "Science ruled supreme" (p. 52). In 1930, the NAFC changed its name to the National Shellfisheries Association and broadened its membership eligibility and mission, now encompassing promoting cultivation of molluscs, disseminating information, and discussing the relevant science, economics, and public and private administration of the fisheries at its meetings.

The following year, the young Victor Loosanoff gave his first talk at the NSA convention that was attended by C. M. Yonge and other notable British biologists (pictured in the book). Carriker sketches the history of biological research relevant to the industry by listing the topics and presenters of papers at the NSA annual meetings each year during this period, as the broadened NSA and OGDA continued to meet together during the 1930s and 1940s. In 1942, the young Mel Carriker presented his first scientific paper, on how the oyster drill *Urosalpinx* penetrates the oyster's thick shell, before joining the U.S. Navy at war.

The tradition of melding science and industry continued to thrive during the post-World War II period, and Carriker quotes later NSA president Albert Sparks as stating that "the long-time tradition of the trade Association and the scientists involved in most of the research on oyster resources was unusual, if not unique, in commercial fisheries" (p. 106). Mel Carriker served as President of the NSA in 1957-1959 and he started its *Proceedings*. Now the *Journal of Shellfisheries Research*, it has ascended to prestigious international status, mainly under the leadership of Sandra Shumway, and is now ranked in the top half of journals in marine and freshwater biology.

The latter part of the 20th Century saw rapid changes in both biological studies and commercial use of shellfish. Techniques in marine aquaculture evolved rapidly. Artificial propagation became routine, and additional species—clams, mussels and scallops—were successfully cultured. The 1987 NSA meeting, the first in 56 years independent of OGDA (now the Shellfish Institute of North America or SINA), featured three special sessions, all organized by women scientists. These treated toxic algal blooms, the biology of *Busycon*, and selection and breeding for bivalve aquaculture. Until the mid-1960s the proportion of female authors of presentations at NSA meetings had hovered around 5%. It then began a still rising curve and is presently at about 45%. And the first two NSA presidents of the young 21st Century have also been women.

Research facilities, now largely under NOAA, also increased apace. The Milford Laboratory facility is now seven times as large as when Loosanoff retired in 1967, and a new 49-foot vessel, appropriately named the *Victor Loosanoff*, arrived in 2004. It replaced the venerable *Shang Wheeler*, which served the Laboratory for half a century and also supported many cruises of Yale faculty and graduate students (including this reviewer), surveying the benthos of Long Island Sound.

In her preface to the book, present NSA President Sandra Shumway aptly characterizes Mel Carriker as "a pearl in the oyster of life" His book chronicles a professional society's services to its membership as well as its trials and tribulations over nearly a century. Beyond that, he documents the unusual and sometimes uneasy mutualism between a primarily biological society, the NSA with both basic and applied aims, and its industry counterparts in the OGDA-SINA, for more than a half century (1930-1986).

The oyster has been tamed—may the author have many more wild years!

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